LA-UR- 03-4705

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Title: DEVELOPMENT AND POTENTIAL OF CRITICAL FLUID TECHNOLOGY IN THE NUTRACEUTICAL INDUSTRY

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Submitted to: DRUG DELIVERY AND SUPERCRITICAL FLUID TECHHNOLOGY. P. York, U. B. Kompella and B. Shekunov (eds.), Marcel Dekker, New York

(Submitted to Drug Delivery in Supercritical Fluid Technology – June, 2003

DEVELOPMENT AND POTENTIAL OF CRITICAL FLUID TECHNOLOGY IN THE NUTRACEUTICAL INDUSTRY

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I. INTRODUCTION

I.

Nutraceuticals, as the name suggests, are ingested substances which combine the benefit of food nutritional requirements, while offering some aspect of therapeutic protection to the human body. Such foods and natural substances are called functional foods, designer foods, pharma foods, as well as many less elegant descriptors. Functional foods are similar in appearance to conventional foods, are consumed as part of a normal diet regime, and have demonstrated physiological benefit, i.e., reducing the risk of a disease state. Naturally-derived products are purchased to enhance stamina and energy, for weight control, to avoid illness, and to compensate for the lack of exercise. Depending upon the definition of a nutraceutical, the market ranges of such products is conservatively estimated to be 3.15 - 4.6 billion dollars in the USA and range from 1.05-1.6 billion US dollars in Europa. A broader definition of Afunctional@ food pegs their US market value between 14.2-17.6 billion US dollars, and if one assumes that 50% of the food selected for consumption is based on health or medical considerations, then the estimated value of the nutraceutical market expands to 250 billion US dollars [1].

Consumers of nutraceuticals have expressed concern about pesticide or chemical residues, processing technology that contributes to ecological pollution, antibiotics or growth hormones in their foods, and the extensive use of preservatives in the foods they consume. It is for these reasons that technologies incorporating the use of critical fluids become important in the production of nutraceutical ingredients. Critical fluids, such as carbon dioxide (SC-CO_2), SC-CO_2/ethanol mixtures, and subcritical water, are environmentally benign processing agents; leaving no solvent residues in the final products, while minimizing the oxidation or degradation of thermally-labile components. Even a cursory inspection of the common classes of nutraceutical agents: herbs, specialty oils, plant extracts, specific protein

fractions, and antioxidants, suggest a link between the two fields.

Table 1 lists some of the common and popular nutraceutical agents in use today, their application, as well as the use of critical fluid processing for their production. It should be noted that all of the nutraceuticals listed in Table 1 can be processed using critical fluids, however a yes indicates that actual production of the nutraceutical components has commenced. Indeed, a segment of the production capacity of the over 50 critical fluid processing plants worldwide are devoted to producing products for the nutraceutical market.

Critical fluid processing can be used in several modes for producing nutraceutical ingredients or functional foods. Exhaustive extraction in which SC-CO_2 or a SC-CO_2-cosolvent mixture is used to yield an extract equivalent to those obtained with organic solvent extraction or via pressing/expelling technologies [2], as documented in the recent literature [3]. Fractional extraction where extraction pressure, temperature, time, or the addition of a cosolvent is varied on an incremental basis, is also capable of producing extracts that are somewhat either enriched or depleted in the desired nutraceutical agent [4]. Such fluid density-based or cosolvent-assisted extractions frequently yield extracts with considerable extraneous material; indeed specifically extracting or enriching a desired solute out of natural product matrix is somewhat akin Ato finding a needle in a haystack@. The problem is shown in Table 2, where many of the listed naturally-occurring oils have been extracted with SC-CO_2, however the targeted Anutraceutical@ components in the right column occur in these SC-CO_2 extracts at very low concentration levels.

To enrich the concentration of desired component(s), researchers have resorted to fractionation techniques utilizing critical fluids. One of the simplest techniques separates the extract with the aid of multiple separators placed downstream after the main extraction vessel. These separator vessels are held at different combinations of temperatures and

pressures [5]. Using such an approach, the fractionation of essential oils from waxes and oleoresins can be accomplished. The use of fractionation columns in which a temperature gradient is imposed on a solute-laden flowing stream of SC-CO_2, either in a batch or countercurrent mode, can also be used to fractionate supercritical fluid extracts. This technique has been used for the production of fish oil concentrates [6], fractionation of peel oil components [7], and mixed-glyceride fractionation [8]. The coupling of critical fluids with chromatography on a preparative or production scale offers another alternative route to producing nutraceutical-enriched extracts. These chromatographic-based separations range from simple displacement or elution chromatographic schemes, i.e., for the removal of cholesterol [9], to the more sophisticated simulated moving bed technology [10]; the latter technique perhaps more favored for the purification of pharmacological compounds.

Although there are many examples for processing nutraceutical ingredients using critical fluids, we will discuss here specific methods for producing extracts, fractions enriched in nutraceutical ingredients, i.e. products containing sterols and sterol esters from natural sources. Sterol esters have been clinically-evaluated [11], and proven to effective for inhibiting cholesterol absorption and synthesis in the human body. This has resulted in the marketing of two commercial products, ABenecol@ and ATake Control@ on a worldwide basis [12]. Likewise, nutraceuticals containing tocopherols or phospholipids, can be readily produced via critical fluid technology as will be described shortly. In addition, an alternative route for producing "naturally" synthesized nutraceutical components, such as the above sterol esters, or transesterified esters can be accomplished in SC-CO_2, particularly if enzyme catalysts are used. Also amenable to this type of synthesis are the "omega -3" fish oil type of esters which are highly valued and an article of commerce in the nurtraceutical field.

II. THE RATIONALE FOR USING CRITICAL FLUIDS

The use of critical fluids for the extraction and refining of components in natural products has now been facilitated for over thirty years. Early success in the decaffeination of coffee beans and isolation of specific fractions from hops for flavouring beer, using either supercritical carbon or liquid carbon dioxide, are but two examples of the commercial application of this versatile technology. Critical fluid technology, a term that will be used here to embrace an array of fluids under pressure, has seen new and varied applications which include the areas of engineering-scale processing, analytical, and materials modification.

However, beginning in the early 1990's, an awareness of the potential of critical fluid processing as a viable component of the newly-coined term, Agreen@ processing arose [13]. This coupled with an increasing consumer awareness of the identity and use of non-green chemical agents in food and natural products processing, suggested a promising future for the use of such natural solvents as supercritical fluid carbon dioxide (SC-CO_2), ethanol, and water. Indeed, labels on food products and ingredients which tout, Anaturally decaffeinated@ and Anature in its most concentrated form: high pressure extraction with carbon dioxide@, have an appeal to the consumer who is aware of food safety issues. Recently, certain nutraceutical products have been labelled as Ahexane-free@, to alert the general public to the undesirable use of organic chemical-based processing agents.

There is a certain synergy that exists between critical fluid processing and the use of nutraceuticals Consumer use and acceptance of these natural products is heightened by the appeal that they have been Anaturally processed@. However this is not a new development and it is interesting to note that many of today=s nutraceutical components have already been extracted or separated using the above-named natural agents. In addition, critical fluid

processing has also been used to create healthier and functional foods, such as: eggs with a reduced cholesterol content [14], low fat nut products [15], pesticide-free natural products [16], spice extracts [17], as well as cholesterol reduction in meats [18]. There is no doubt that the development of such products coupled with a proper commercial marketing campaign provides a powerful stimulus for the consumer to try these products.

III. A GREEN PROCESSING PLATFORM

As noted previously, SC-CO_2 reigns supreme as the principle processing agent in critical fluid technology. However this situation is changing, particularly with the recognition that SC-CO_2 cannot be effectively utilized for all tasks, and is a relative poor extraction solvent for polar compounds. For certain applications, the addition of a minimal amount of cosolvent (usually an organic solvent having a higher T_c than CO_2), suffices to improve the extraction of specific components from a natural product matrix. However the number of GRAS (Generally Recognized As Safe) cosolvents is rather limited (e.g., ethanol, acetic acid). Such cosolvents can be used in conjunction with SC-CO_2 under conditions which can favour the formation of a one or multi-phase extraction medium, capable of producing the desired end result.

Other processing media which embrace the green processing concept are the use of liquefied gases (e.g., LCO_2) and certain liquids under pressure above their boiling point. By the application of external pressure, liquids such as water and ethanol can be used above their boiling point. Such liquids can be used to produce equivalent or superior products compared to those derived by using conventional liquid solvent extraction [1999]. For example, St. John's wort active components can be more effectively isolated using critical fluids, and/or by using SC-CO_2 along with subcritical water [20]. Recently, the author and his colleagues have utilized subcritical water between 120 – 160 ^{o}C to isolate anthocyanins from natural

berry substrates [21]. In the processing of natural products, optimization of the extraction temperature is important in order to avoid degradation of the extracted components, such as the anthocyanins. For the extraction of anthocyanins, 120^{o}C proves to be the optimal temperature. Liquefied carbon dioxide, LCO_2, has also been used at near- or sub-ambient temperatures [22, 23], thereby avoiding conditions which are conducive to thermal degradation of the extracted components.

Subcritical water complements SC-CO_2 for the environmentally-benign processing of nutraceuticals. Its phase diagram (Figure 1) is similar to that of CO_2, although the critical temperature and pressure of water are much higher (T_c = 374^{o}C, P_c = 221 atm) than those for CO_2. However the region in the phase diagram that is of interest for processing nutraceuticals lies between 100 - 200^{o}C, in the liquid region defined in Figure 1, and at pressures less than 100 atm. Recent studies have shown that subcritical water under these conditions can be effective for extraction of the natural products, such as cloves [24] and rosemary [25].

A considerable literature exists on the properties and use of water in the superheated state, and its temperature has been shown to be a key parameter for regulating the solvent power of superheated water [26]. The dielectric constant of water varies inversely with temperature, where it varies from 30-60 over a temperature range of 100 – 200 ^{o}C. Dielectric constants of this magnitude are in the range of those exhibited by polar organic liquids. Therefore subcritical water can be potentially used as a substitute for less-desirable organic solvents, including ethanol, to extract and process natural products.

Figure 2 summarizes the above in terms of offering an Aall natural@ approach to processing natural products for nutraceutical ingredients. On one end of the solvent scale lies SC-CO_2 and LCO_2, while pressurized water on the other end is available for isolating polar moieties. Several combinations of GRAS cosolvents can be coupled with CO_2 for cases

where this approach proves viable. Sequential processing of a natural substrate by the use of these various fluids is also possible as suggested by the hypothetical scheme noted in Figure 3 for soybeans as a natural substrate. Here the non-polar components, such as carotenoids, triterpenes, or phytosterols, are preferentially removed by carbon dioxide followed by extraction with a CO_2/cosolvent combination that can remove the more polar components, such as phenolic acids or coumarins. Finally, after removal of the above components, subcritical water can be applied to isolate the isoflavones, phytates, etc. It should be recognized that some of targeted nutraceutical compounds may occur in each of these Agreen@solvent combinations. An additional benefit from the process depicted in Figure 3 is that left over residual proteinaceous meal is available for further use, devoid of any solvent residues. This is an appealing extraction and/or fractionation scheme that can be accomplished using the same high pressure processing equipment, as has been noted by King [27].

IV. TECHNIQUES FOR ENRICHING AND ISOLATING NUTRACEUTICALS

The development of critical fluid-based techniques for isolating nutraceutical ingredients requires sequential development in steps of increasing complexity and scale up. These are summarized as follows:

Bench Scale SFE Evaluation of Process Feasibility

Establishment of the Need for Fractionation

Pilot Plant Evaluation

Scale up to Plant Stage

Bench scale evaluation of the feasibility of extracting a nutraceutical is usually accomplished with the aid of a small scale extractor. Such units are available commercially, but an

increased experimental flexibility is achieved by constructing a customized SFE unit. Alternatively, there exists the availability of small scale SFE equipment, normally intended for analytical uses of critical fluid technology, which can also be used to optimize and assess the extraction of a natural product [28]. Both of the above approaches can also incorporate the introduction of a cosolvent into the critical fluid, should this be required in the processing of a nutraceutical source.

Enrichment of a nutraceutical ingredient to a sufficient purity or concentration cannot always be accomplished by using just an extraction step, and this will necessitate the use of a fractionation method, in one of several forms; or even by combining two or more fractionation methods. A combination of mixer/settler modules is one mode of amplifying the effects of single stage SFE [29]. A more popular approach for fractionating natural products is the use of a fractionating tower with internal packing, and that frequently will incorporate a temperature gradient along its length [30]. Introduction of the material to be fractionated can be made at the bottom, top, or an intermittent position in the column. Both co-current and counter current operational modes can be enacted with respect to contacting the critical fluid with the substrate whose components need to be separated. Chromatographic fractionation has also been utilized [31] including the use of simulated moving bed technology [32]. Examples of these chromatographic options will be discussed later.

The pilot plant evaluation stage incorporates both a scale up of the previously described approaches as well several other processing options. Although somewhat rare, single pass (with respect to the extraction fluid) pilot and production plants are known to exist, but plants designed for fluid recycle with integrated heat exchangers are much more the norm. Batch pilot and production scale plants have been in use for some time, and several

novel approaches have been described which allow the continuous feed of substrates into extraction or fractionation units, i.e., lock hoppers, augers, etc. {33].

Final scale up to the production plant stage is a serious undertaking, both with respect to mechanical complexity, safety, and economics. Such plants have traditionally been more or permanent in design, however there is a trend toward increased flexibility (e.g., multiple use modules for extraction, fractionation, reaction), considering the initial investment in the in common pumps and fluid sources. Portable extraction units have also been constructed and their feasibility demonstrated for the field side processing, thereby permitting extraction of natural products, immediately after they are harvested. This avoids degradation during the transport of the nutraceutical ingredient, i.e , medicinal ingredients in chamomile.

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A. Examples of Experimental/Processing Equipment and Methods

To describe some of generic approaches mentioned in the previous section for processing nutraceuticals; several examples are provided in this section to illustrate the equipment which is appropriate for conducting extractions and fractionations with critical fluids. Figure 4 shows a schematic of a bench top extraction system that has proven to be very versatile in our laboratories. A fluid source, A, (e.g., CO_2), which can be can be either gaseous or liquefied, is of course required. Use of liquefied CO_2 has the advantage of being more compatible since this is what is found most frequently in use, on scaled up equipment. A compressor or liquid pump, C, delivers the fluid through a tandem switching valve, SV-1 and SV-2, to a tubular extraction vessel cell, that is held at a constant temperature to maintain the fluid in its desired critical state (sub- or super-critical). The fluid than passes over the material in the extraction vessel and is routed through another switching valve arrangement. In Figure 4, either a micro-metering valve, MV; or back pressure regulator; is used to reduce

the pressure on the fluid as it exits the extraction/fractionation stage.

The receiver vessel can be of several formats, but will frequently be packed with internal packing to eliminate entrainment of the extracted components form the rapidly expanding critical fluid. Flow conditions under ambient conditions are measured with the aid of a flow meter, FM, and fluid totalizing module, GT. This described SFE unit can be changed to accommodate fractionation and reaction experiments involving, as has been shown and documented in our laboratories at a modest cost [34]. Such equipment today exists commercially, but may be more limited with respect to pressure range, fluid flow rate, and size of the extraction chamber [34].

An increase in processing capability to a mini-pilot plant stage is shown in Figure 5. This unit incorporates a fluid recycle option which reduces substantially the mass of extraction fluid required for SFE, since the initial charge of extraction fluid is used continuously throughout the extraction. The four liter extraction vessel permits multi-kilogram quantities of natural product to be extracted. For this vessel, and others utilized for conducting extractions up 70 MPa (680 atm), a safety factor of 2.5 times the maximum operational pressure is specified when ordering these vessels. Back pressure or metering valve arrangements used for pressure reduction of the extraction fluid are heated to overcome the attendant Joule-Thomson cooling effect as the depressurized fluid is jettisoned to the collection vessel.

The collection vessel must be sufficient size to allow separation of the extracted solutes from the depressurized fluid. For these purposes a 2-liter collection vessel is utilized, as shown in Figure 5. By following the vector arrows in Figure 5, one can trace the depressurized fluid flow path back to the compressor where the fluid is re-pressurized to continue the extraction. A sorbent-laden column maybe inserted in the low pressure side of

this scheme for purifying the exhausted fluid of any unwanted odoriferous volatile compounds. A provision for makeup of fluid lost upon draining the extract from the receiver vessel is noted in Figure 5 as the ACO_2 makeup@.

A larger semi-continuous pilot plant [35] is shown in Figure 6. It consists of three, 4-liter extraction vessels, arranged with an appropriate valve sequence to permit several modes of operation. However the principle feature that distinguishes if from the smaller unit noted in Figure 5, is that it allows for semi-continuous processing using the tandem vessel arrangement depicted in Figure 6. Here carbon dioxide, the extraction fluid, can be routed sequential to one or more of the extraction vessels, which can be operated so that one of the vessels, A, is being extracted while another vessel, B, is being loaded with product to be processed, and a third vessel, C, is capable of undergoing pressurization/ depressurization. That these operations can be accomplished in parallel is also apparent, lending the unit to semi-continuous operation, even for the processing of granular solids.

Other types of pilot plants, including commercial units embracing this principle are available. Some selected vendors of pilot plants; although this is not an exclusive list, include, UHDE, Thar Designs, Applied Separations, Chematur, and Separex.. Likewise, bench scale equipment for preliminary evaluations are manufactured by such companies as Autoclave Engineers (now called Snap-Tite), Chematur, Nova Swiss, Applied Separations, Nova Sep, Thar Designs, Pressure Products, Inc., Supercritical Fluid Technologies, and Separex.

In the future, extraction and other processing options may be accomplished with the aid of an expeller, i.e., the critical fluid component will be introduced into the expeller barrel. This mode of processing not only allows extraction/fractionation to be accomplished on a continuous feed of raw material, but allows the introduction through selective dissolution in

the fluid, of nutraceutical ingredients, permitting them to be naturally Aimpregnated@ into a product matrix. The addition of CO_2 into the barrel of the extruder, where it becomes a supercritical fluid due to the heat and pressure generated during the extrusion process, not only facilitates solubilization of materials from the substrate being processed, but also enhances the fluidity of the potential extract e.g., a nutraceutical based oil. Two active companies in this field are Critical Processes Ltd. in North Yorkshire, England, and Crown Iron Works in Minneapolis. The former firm is focussed on nutraceutical extraction, citrus oil deterpenation, etc.; while the latter company is concerned with CO_2-assisted recovery of oils from seeds.

B. Fractionating with Critical Fluids

The ability to fractionate naturally-derived materials in a benign way using critical fluids is of particular interest to processors of nutraceutical ingredients. This is due to the fact that to obtain useful extracts, an enrichment or isolation of the more highly purified form of the nutraceutical component increases the value and utility value of the derived- product to nutraceutical manufacturers. Hence approaches to achieve the above result are discussed in this section.

One of the important methods of critical fluid fractionation involves the countercurrent separation of phospholipids from a vegetable oil. A system to achieve this end is noted in Figure 7. Here high pressure carbon dioxide is fed into a pressure vessel packed with segmented gauze mesh packing (the Arefining vessel@ in Figure 7), where it travels upwards contacting soybean oil, which is pumped into the top of the refining vessel using the designated liquid pump. The two media contact one another in the refining vessel, in which oil is solubilized in the SC-CO_2 ; while the phospholipids being insoluble as the

CO_2 descends to the bottom of the refining vessel. The oil can be recovered by lowering the pressure and temperature in the receiver vessel allowing recycle of the CO_2 back to the main compressor. By using this technique, an extract enriched in lecithin precipitate can be isolated without the use of organic solvents [36].

A somewhat more sophisticated fractionation method involving the use of vertical packed fractionating towers is currently being applied to enrich nutraceutical ingredients from liquid natural feedstocks. The liquid to be fractionated can either be fed concurrently or countercurrently into the fractionating column. There are certain advantages in terms of fractionation efficiency that are provided when using the countercurrent mode and by introducing the liquid feed into the center of column, thus creating extraction and raffination sections. An example of a fractionating column operated in the concurrent mode is shown in Figure 8. For experiments conducted in the author's laboratory, the unit is usually operated in a batch mode, but can be made to operate on a semi-continuous basis, using a liquid pump to feed substrate into the column (see in Figure 8). The components to be separated in the substrate are subjected to a thermal gradient along the length of the fractionating tower, where each of the designated sections of the column, have an increasing temperature for the sequence T2, T3, T4, and T5. This allows fractionation of the components in the substrate feed based not only on their relative solubilities in the decreasing CO_2 density gradient, but also according to the increase in their respective increasing vapour pressures as they ascend the column. This type of fractionation system has been used to deacidify olive oil, deterpenate citrus oils, and to fractionate fish oils or butter fat. The recent approach of Clifford, et al. [24] involving the deterpenation of citrus oils using subcritical water, also applies this fractionation principle.

Chromatographic fractionation using critical fluids as mobile phases has been studied

for some time now, however scale up from the analytical regime has been less prevalent. Studies at the National Centre for Agricultural Utilization Research by King and co-workers [31] have demonstrated that preparative supercritical fluid chromatography (SFC) can be coupled advantageously with a selective SFE enrichment stage to yield concentrates rich in nutraceutical ingredients. As shown in the processing scheme in Figure 9, flaked soybeans are initially extracted at a relative low pressure to enrich the components of interest, the tocopherols. This fraction is then moved sequentially on to a sorbent filled column, for further fractionation to yield a tocopherol-enriched extract of nutraceutical value. The advantage of this approach is it allows one to enrich a particularly valuable nutraceutical ingredient from a natural matrix, without contaminating of the remaining matrix with a noxious agent. Enrichment factors relative to the tocopherol content in the original soybean flakes are tabulated in Table 3. Note that these are only modest tocopherol enrichment factors for application of the single SFE step, however significant enrichment of the desired components can be obtained by than applying than supercritical fluid chromatography for further fractionation and enrichment of the tocopherols (Table 3).;

Recently, using a similar approach, Taylor, et al. [37] have been able to produce concentrates enriched in phospholipids (PPLs) for potential use in the nutraceutical industry. Table 4 summarizes the relative amounts of PPLs via initial SFE isolation and than in the fractions obtained after SFC. Here the major component in the starting substrate (soybean oil triglycerides), were initially reduced in the SFE step using neat SC-CO_2, followed by sequential SFE/SFC utilizing SC-CO_2/ cosolvent mixtures on the lecithin-containing residue which remains after SC-CO_2 extraction.. By using SC-CO_2/ethanol/water fluid phases, one could not only perform preparative SFC for enriching the PPLs, but can also obtain in certain cases individual purified PPL moieties. This is achieved by selective density and

compositional programming of the SFC fluid phase, coupled with time-based collection of eluent fractions.

Mention has been made previously of the use of subcritical water as a “green” processing agent. Studies performed in the 1970's by Schultz and Randall [38] showed that useful fractionations of flavour components could be obtained by using LCO_2 in a countercurrent mode with fruit-based aqueous feedstocks. This approach produced fruit flavour essence concentrates enriched in the more hydrophobic components. Recently a similar method exploiting LCO_2/aqueous phase partitioning has been developed by Robinson and Sims [39] using a microporous membrane to further increase the fractionation efficiency in such systems. This Porocrit fluid fractionation process is depicted in Figure 10 and shows Anear@ critical CO_2 being fed into a concentric tube arrangement, countercurrent to the flow of aqueous liquid feedstock, which is being pumped internally through a microporous membrane. Again an enriched extract exits with the depressurized CO_2, producing an aqueous fruit aroma of the composition described in Table 5. Here the listed components in the feedstock have been concentrated in many cases, over 100-fold with respect to the starting concentrations. Note also that the Porocrit process is very effective at removing the flavor components, as judged by the listed solute depletion data given in the last column of Table 5.

V. THE ROLE OF ANALYTICAL SF TECHNOLOGY IN SUPPORT OF NUTRACEUTICALS

The analytical use of critical fluids spans over three decades of endeavour and applications [40, 41] and there are many excellent tomes describing activity in this field [42, 43]. It is not the intention of this review to provide a detailed description of the use of

analytical methodology for characterizing nutraceutical products, although such techniques as supercritical fluid chromatography, can rapidly provide valuable information for the chemist and product formulator [44, 45].

There is no doubt that analytical SFC is a logical choice to characterize extracts or fractions obtained by critical fluid extraction and fractionation, but perhaps of more importance, is to describe options that exist for utilizing such analytical instrumentation to assist in optimizing and developing processing methods to produce nutraceuticals for the marketplace. A summary of the information obtainable using analytical scale techniques and methodology are given in Table 6.

With the advent of automated analytical SFE equipment, it has become possible to rapid ascertain what extraction or fractionation conditions would be most relevant in scaling up the process. In the United States, analytical SFE instrumentation is produced by such firms as Isco, Applied Separations, Leco, and Jasco. Similarly, analytical scale SFC equipment is available from Berger Instruments, Thar Designs, Jasco, and Sensar. The equipment which can be obtained from these vendors, can be if needed, slightly modified to study the conditions that are amenable to processing nutraceuticals. King [34] has provided a interesting review of how lab constructed equipment can be used for both analytical and process development purposes.

Two examples will be cited that show how analytical SFE instrumentation can be used to obtain information related to isolation of nutraceutical ingredients. Chandra and Nair [46] have studied the extraction of isoflavone components, such as daidzein and genistein., from soya-based products, using a manually-operated SFE system. Neat SC-CO_2 proved relative ineffective in removing the isoflavone components from the various soya-base matrices, however by simply varying the conditions for affecting the extraction, it was found

that 20 volume percent ethanol in SC-CO_2 at 50^{o}C and 600 atmospheres could remove over 90% of the isoflavones in under 60 minutes extraction time. These survey extractions were performed on sample sizes ranging from 2-10 g, saving considerable time and labour in ascertaining what conditions were optimal for the extraction of these components. The need for relative high pressures and cosolvent to remove the isoflavones from the matrix suggest that subcritical water extraction might be another alternative for isolating these nutraceutical components.

An automated SFE option has been utilized by Montanari, et al. [47] (1999) and Taylor, et al. [37, 48] to develop the conditions most amenable to isolating phospholipid concentrates from deoiled soy meal. In the former case, a set of conditions were tested by programming the microprocessor controller of the automated SFE to run a large number of extractions on a common samples at various pressures, temperatures, and cosolvent (ethanol) levels. The results showed that at 70^{o}C and 40.7 MPa with 10 mole % ethanol, that phosphatidylcholine could be extracted preferentially relative to the other phospholipids in the soya meal. By utilizing higher pressures, it was found that the total amount of phospholipids extracted could be substantially increased at the slight reduction of the phosphatidylcholine content in the final extract.

Similarly, an automated SFE unit was also used to ascertain what conditions were necessary to elute and separate phospholipid moieties from extracts obtained from SFE and other extraction methods [37, 48]. In this case, the phospholipid-containing extract was layered on top of an adsorbent, such as alumina or silica gel, and elution and separation of the target compounds assessed by changing the pressure, temperature, and quantity and composition of the cosolvent in the supercritical fluid eluent. As summarized in Table 7, pure SC-CO_2 aided in removing the non-polar (triglyceride) components from the deposited

sample, but the presence of a binary cosolvent with the SC-CO_2 proved necessary to affect elution of the phospholipids from the sorbent bed. By collecting various fractions during the stepwise elution from the column, while varying the eluent conditions, various phospholipids could be enriched as indicated in Table 7. These experiments could be run in several days, and even over night on the automated SFE module, saving considerable cost and effort before scaling up the SFE/SFC process to a preparative level. Using a similar approach, experiments were run on other natural product matrices in terms of ascertaining whether comminuting the sample prior to conducting a SFE was necessary. Alternatively such instrumentation can assist in identifying what part of a natural plant, actually contains the targeted components of interest. [49].

It was mentioned previously that analytical SFC could be of considerable use in evaluating the content of nutraceutical-containing extracts or feedstocks. This is particularly true if one is looking for a rapid analysis method for quality control of selected ingredients, or to monitor qualitative differences in processing and raw materials. The elution order of lipophilic solutes is well known in capillary SFC [50] and permits the separation and identification of key lipophilic nutraceutical components. For example, in Figures 11, the high resolution separation of fatty acids, squalene, tocopherols, phystosterols, and various glycerides contained in deodorizer distillate is shown using analytical capillary SFC using flame ionization detection (FID) [51]. The resultant profile is of a valuable feedstock for nutraceutical components and indicates that its components are soluble in SC-CO_2 (the mobile phase in for this analytical SFC separation), as well as revealing valuable information to the analytical chemist on the chemical composition of the sample. This is an excellent assay method considering the sample preparation is minimal and the analysis time under 45 minutes.

On the subject of sample preparation, analytical SFC can save the analyst considerable time as illustrated by the SFC profile of the composition in a nutraceutical capsule containing saw tooth palmetto berry extract (Figure 12). In this case, the extract was dissolved and diluted with a minimal amount of hexane and directly injected into the chromatograph. By density programming the CO_2 mobile phase, a high resolution chromatogram can be facilitated. The complexity of the saw tooth palmetto berry extract is apparent and consists of a mixed composition of fatty acids and trace sterol components. Recently a similar approach has been used by others for characterizing saw palmetto extracts [52].

VI. PRODUCTION PLANTS AND NUTRACEUTICAL PRODUCTS

The purpose of this section is to provide a brief overview of the magnitude and scope of critical fluid technology as applied to the production of industrial products, including nutraceuticals. Information on the use of critical fluid production facilities can be limited due to proprietary constraints, however production processes using supercritical fluids have existed for over thirty years, and critical fluid processing can no longer a novelty industry. These production facilities vary considerably in the magnitude of the operation, ranging from the large Houston-based decaffeination plant of General Foods, to smaller extraction facilities focussed on the production of non-commodity items. Aside from decaffeination plants, there is a sizeable segment of the production facilities devoted to the processing of hops. However these facilities are devoted to hops processing for only part of year and potentially have additional capacity which could be devoted to processing of materials having nutraceutical value.

There are over 50 plants (not pilot plants) worldwide devoted to critical fluid processing. Currently most of these are located in Germany, the United States, France, and Japan. Other nations, such as Britain, Australia, India, and Italy continue to develop more capacity for critical fluid processing; and other nations with a rich litany of natural products, will undoubtedly enter the marketplace as users. Table 8 shows a list, which is not inclusive, of many users of critical fluid extraction for food and natural product processing throughout the world. Key players in identified market segments are: decaffeination - General Foods, SKW Trostberg, Kaffee HAG, Hermsen; hops processing - HVG Barth ($NATECO_2$), John Haas, Yakima Chief, Carlton United Breweries, Steiner Hops, English Hops, SKW Trostberg; flavors/spices - Cultor, Quest, Flavex, Norac, US Nutraceuticals, Ogawa, Fuji Flavor, Kobe, Mori Oil Mills, Takeda. Many of the processors listed under the generic heading of flavors/spices do a variety of other natural products such as specialized oils, natural pigments; e.g., Flavex is involved in the processing of ginseng among other moieties. As noted above, many of these companies have additional capacity for fulfilling the needs of the nutraceutical market and other firms, on a more diminutive scale, and are preparing to enter the market focussing on the processing of niche natural products. Some of the new companies addressing nutraceuticals processing specifically are US Nutraceuticals, KD-Pharma - IQA, Wells Investments Ltd., Aromtech – OY, GreenTek 21, and Arkopharma.

There also exists a cadre of companies that specialize in research and development and toll refining up to a certain scale. Among the more prominent ones are Phasex, Praxair, Norac, Marc Sims Inc., Separex - Hitex, Flavex, Critical Processes Ltd., Bradford Particle Design (BPD), CPM Inc., Wells Investments Ltd., and Aphios Corp. These companies and a host of other organizations; consultancies, academia, and government laboratories, can be useful sources for technical consultation/information.

What is a typical extraction/processing plant like in terms of scale? This is hard to quantify without picking what might be a Atypical@ example, but it is worth citing the $NATECO_2$ production facilities in Wolnzach, Germany as an example of a technically sophisticated and diverse operation. Some of their stated capacity and capabilities are as follows:

Production Plant #1 - 4 X $2m^3$ Extraction Vessels

Laboratory Plant #2 - 1000 mL Counter-current Column

Production Plant #3 - 4 X $4m^3$ Extraction Vessels

Production Plant #4 - 3 X 500 L Vessels

Pilot Plant #5 - 2 - 200 L Counter-current Column

This plant has historically focussed on the processing of hops, but has diversified over the years to include the processing of other natural products, thereby providing for maximum utilization of the plant facilities.

To enumerate and discuss all of the nutraceutical, or potential nutraceutical products that could be processed by critical fluids is beyond the scope of this review, particularly when one considers that in the past, hat many of these naturally-derived products have been extracted with critical fluids. However it is worth noting how these extracts are usually obtained in the SFE mode and what generic classes of materials have been extracted. For example, it is rare to obtain an extract without the occurrence of lipid co-extractives, hence the nutraceutical agent will frequently be diluted in a oily matrix. Examples of this type of extract are the previously mentioned tocopherol-containing extracts as well natural pigments, such as the carotenoids [53]. Tocotrienols can also be isolated via SFE as a complex from barley brand; likewise the newer sterol or steryl ester concentrates can be obtained as mixtures from corn oil, rice brand oil, or saw palmetto sources. Many of these extracts containing other helpful nutraceutical ingredients, such as fatty acids, squalene, etc., that can

act in a synergistic mode with the principle nutraceutical agent.

It is worth noting that many current oil extraction and refining methods remove valuable nutraceutical components from the oil in the name of appearance and flavor. By-products and streams from these milling and refinement steps often contain pre-concentrated sources of nutraceuticals, e.g., deodorizer distillate, residual protein meals, fibrous materials. Sources such as corn gluten meal, corn brand fibre and alfalfa leaf protein concentrate have been extracted with SC-CO_2 successfully for their pigment, sterols, and fatty acid content. The high tocopherol-sterol-squalene content of deodorizer distillate is well known and several schemes employing critical fluids have been reported for fractionating this material to achieve higher purity materials [54]. Another processing agent that is a concentrator for nutraceutical components are the bleaching sorbents used by the vegetable oil processing industry. King and co-workers [55] have shown that very high oil yields can be obtained from clay bleaching earths, however there is a need for the resultant extract to be analyzed for enriched content of the nutraceutical components.

Critical fluid extraction has been applied for sometime now in the extraction of speciality oils, such as evening primrose, borage, blackcurrant, and flax. These moieties contain the presence of gamma-linoleic acid, a component which has been implicated favourably for the treatment of several medical conditions. Other specialized oils that have also been extracted with SC-CO_2 are wheat germ, avocado, sea buckthorn, sorghum brand/germ oat, and amaranth. There has been recently some studies employing critical fluids to obtain oil from fungi or marine sources, like spirulina, which are devoid of cholesterol. Partial deoiling has also been performed using SC-CO_2, more with respect to developing a functional food ingredient that has less fat (oil) or cholesterol content, i.e., low calorie peanuts. Such deoiling can be done using either SC-CO_2 or propane, and still meet

the criterion or use in functional food products.

As has been stated previously, phospholipids and many of the traditional herbal medicine type components are amenable to critical fluid extraction provided that GRAS cosolvents are employed along with SC-CO_2. Pure phosphatidylcholine and phosphatidylserine are finding widespread use, the latter in improving cognitive function, hence the challenge for those using critical fluid processing for phospholipid recovery is to develop purification techniques for these naturally-derived chemicals. Other nutraceutical agents that can be obtained similarly include extracts from chamomile, paprika, feverfew and gingko biloba (analytical studies), garlic, and ginger. Most of the common spices, mint oils, including a commercially-available extract of rosemary, can be obtained via SC-CO_2 extraction.

For the products mentioned above, critical fluid technology faces stiff competition from molecular (vacuum) distillation techniques, a time-honoured technique; although greater selectivity is potentially available utilizing critical fluid-based methods. This coupled with the fact that CO_2-derived extracts exhibit is many cases extended shelf lives due to the prophylactic action of the residual, non-oxidative CO_2 atmosphere; as well as microorganism destruction due to exposure at higher pressures and temperatures, argues for a bright future for critical fluid technology in the nutraceutical marketplace.

VII. IMPROVEMENT OF NUTRACEUTICAL FUNCTIONALITY THROUGH PROCESSING WITH CRITICAL FLUIDS

It is obvious from the discussion in the previous sections that many nutraceutical components or compositions are soluble to varying degrees in critical fluid media, particularly SC-CO_2. However unlike pharmaceutical compounds, which are usually highly purified pure solutes dissolved in SC-CO_2 and/or cosolvent mixtures, nutraceutical

components and compositions tend to be ill- defined and molecularly complex mixtures, where often components interact synergistically to induce a therapeutic benefit. Testimony to this complexity is provided by the capillary SFC profile of saw palmetto berry extract provided in Figure 12, where the predominate fatty acid mixture along with low levels of sterols help alleviate prostate conditions in men. Obviously, saw tooth palmetto berry extract is quite different than a high purity pharmaceutical, where perhaps only one or two (i.e., chiral racemates) major components are quantified by chromatography.

As noted in the introduction, the strategy in the nutraceutical or functional food market is to fortify specific foods or natural products with ingredients that have, or are perceived to have, health- promoting benefits. Many of these products are orally ingested, and like pharmacological drugs, can be made more rapidly effective by rapid dissolution within the body. Therefore it behoves us to consider the benefits of producing nutraceuticals by the processes employed to make small diameter particles of medicinally-active compounds for the pharmaceutical industry using the critical fluid-based processes described early in this book This can incorporate techniques like GAS, RESS, PCA, etc. to produce small particles having low polydispersity, or encapsulation of nutraceutical ingredients, and impregnation of an active ingredient into a food matrix, i.e., to make a functional food.

Although this would appear to be a good strategy, it is important to consider economics as they relate to the difference between the pharmaceutical and food industries. The food industry tends to be a commodity-driven marketplace with a low return margin of return on its products. The large hourly production rates which are characteristic of the food industry are a challenge given the current state of the art of particle formation technology employing supercritical fluids. However consumers of nutraceuticals and functional foods tend to be willing to purchase these (nutraceutical) more expensive products, so it is not unreasonable to consider the benefit of applying critical fluid-based techniques to produce

ingredients via the methods noted in the previous paragraph. For example, consider the case for winning lecithin, or a more highly purified form of phospholipid concentrate from soybeans. Isolating lecithin from soybean oil or meal can yield a nutraceutical-grade lecithin that sells for $31 per pound. This is a significant mark up versus the price of soybean oil which might be 25 cents a pound. Hence when one considers a nutraceutical-grade phospholipids concentrate (concentrated phosphatidycholine or phosphatidyserine), the price is $1500/lb. Obviously with these economic incentives, the possibility of applying critical fluid-based particle production processes may be justified.

The fundamental principles and methodology of producing fine particles, encapsulates, etc. using critical fluid processing have been covered in depth in previous chapters, and it is not our intention to cover this as we conclude this chapter. However it is worth considering a few of the possibilities in a generic sense, and examining one application of the technology to a specific nutraceutical component or product. Table 9 lists applications of critical fluid technology reported at the 6th International Symposium on Supercritical in 2003, which have implications for the nutraceutical and/or functional food industry. As one can see, a number of the processes described in previous chapters have been applied to foodstuffs, nutraceutical ingredients or a suitable surrogate (e.g., cholesterol for sterols or sterol esters), or delivery systems that utilize liposomes or biodegradable natural polymers. By controlling the particle size, morphology, of regulation of delivery (encapsulation), these critical fluid-based processes can produce a pleathora of materials as noted in the application column. This includes specific chemicals which are utilized in nutraceuticals, such as high antioxidant containing spices, sterol esters, carotenoids, phospholipids, and fatty acids. This is ample evidence that such processes could impact on the functional food industry, as well as provide new food materials for integration into the conventional food product chain.

We noted previously the high value and application of phospholipids-containing

materials, like lecithin or phospholipid-base liposomes, as functional food ingredients. This is worth examining in greater detail, starting with the historical research of Quirin and Eggers Germany [16, 57, 58] involving the jet extraction of lecithin from soybean oil. These researchers developed a continuous method for deoiling lecithin obtained from caustic or physical refining –derived lecithin feedstocks that resulted in powdered extracts; somewhat analogous to the lecithin- or phospholipid-based powders noted in Table 9. this process overcame some of the physical difficulties in refining lecithin by SFE with SC-CO_2 or SC-CO_2-csolvent mixtures, due to the gelatinous nature of the lecithin feedstock. Further, the method referred to as "jet extraction" provided a more environmentally-benign process than using propane for deoiling lecithin feedstocks.

The basis of jet extraction is that it facilitates the dispersion of a thin filet of lecithin into a highly turbulent jet of SC—CO_2. This is made possible by the use of two overlapping capillary jets. The lecithin is fed into the inside capillary, while the CO_2 enters into the larger outside capillary encasing the smaller diameter capillary tube. The compressed carbon dioxide than mixes with the lecithin extrudate in a mixing chamber under conditions of high turbulence affecting deoiling of the lecithin and formation of a powdered product. The physicochemical basis of this separation process in terms of its effect on lecithin viscosity and interfacial tension have been reported by Eggers and coworkers [57, 58], and resulted in the conceptualization of a continuous processing plant based on the above principle. Other processing options for lecithin with critical fluids are nicely summarized in Stahl's et al. book [16].

A device similar in principle to the German researchers has been described by King [34] as shown in Figures 13 and 13a. Figure 13 shows the general and initial design of the jet extraction system, while Figure 13a provides more critical details. In this laboratory-scale apparatus, the solids collection reservoir and two collector vessels were each 30.5 X 2.54 cm,

316 stainless steel tubing. The lecithin sample to be extracted is placed into the solids reservoir and extruded into the jet tube assembly with the id of a nitrogen pressure head. This "pusher" gas flow rate is regulated by a micrometering valve.

As described above, the SC-CO_2 interfaces with the lecithin sample in the jet tube assembly (Figure 13a). It is critical that in the pictured three-way valve that the viscous sample be injected through the 0.16 cm capillary into the larger concentric tube to avoid viscous back streaming and to assure intimate contact with the SC-CO_2. The solubilized oil components are then routed through the back pressure relief valve, where the CO_2 decompression occurs, resulting in the precipitation of the oily constituents in the liquid collector. The deoiled lecithin powder than drops into solids collection vessel. Careful control must be exercised over the relative flow rates of the pushing and extraction fluids so as to maximize the contact time between the lecithin and the extraction fluid. This can also be amplified by using longer extraction chambers which provide a long contact, or drop time, of the lecithin in the compressed CO_2 atmosphere.

Other notable advancements have been reported in the literature which use critical fluids for producing micron-size, phospholipids-based powders as well as the formation of PPL-base liposomes. For example, Castor at Athios Corporation has patented several concepts (the CFL Process) [59, 60] which permit the encapsulation of hydrophobic drugs as well as naturally-derived drugs, such as taxol. The basis of these patents are that PPLs deposit out at the phase boundary as the PPL-drug-aqueous phase (or multi-lamellar vesicle) undergoes decompression in a critical fluid atmosphere. The resulting liposomes formed in the presence of SC-CO_2 and other alternative fluids showed stability life times exceeding six months, and this could be amplified by the inclusion of ά-tocopherol in the PLL matrix, which provides prophylactic protection in an extending the lifetime of the resultant liposome.

Similar studies have also been performed by the research group of Charbit in

Marseilles [61, 62] in which fine PPL particles were formed by decompression using the SAS process. The focus of this research was to develop drug delivery systems, but it would equally applicable to the functional food area. Typically a two weight percent solution of soy-derived lecithin is dissolved in an ethanol solution which is subsequently injected into SC-CO_2. Typical precipitation temperatures and pressures were 35^{o} C and 8-11 MPa, respectively. The micronized PPL particles tended to be in the range of 15-60 :; were amorphous in nature, and coalescenced when they were exposed to air.

Other schemes have been cited in the literature which employ phospholipids-based materials and supercritical fluids for fine particle formation or encapsulation. Weber et al. [63] recovered lecithin from egg yolk extracts and induced crystallization by implementing the GAS process. Likewise Frederiksen et al. [64] developed a new method of preparing liposomes to encapsulate water-soluble substances with the aid of ethanol. In this study, liposomes could be formed from phosphatidylcholine having 40-50 nanometer dimensions. A mixing process (ESMIC Process) [65] involving supercritical fluids has also reported, which is conducted in a stirred autoclave, to provide embedded pharmaceutical preparations in a lecithin matrix. Final particle size is partial control by the milling process taking place in the stirred autoclave contained the supercritical fluid medium.

In summary, the above studies and processes show the versatility of utilizing critical fluid media for modifying a target nutraceutical for functional food use. In the case of PPLs, the above studies illustrate how they could be prepared for rapid dissolution in food formulations or to encapsulate them for sustained delivery rates, or alternatively, to use PPL-based liposomes, etc. to encapsulate nutraceutical ingredients. Obvious extensions to other target nutraceuticals follow and application of the technology outside of its traditional niche in the pharmaceutical industry seemed assured.

VIII. SUMMARY

In summary, we have attempted in this review to provide some understanding of the basic concepts involved in the use of critical fluids, and to explain how these fluids are now exploited for the production of nutraceutical and other naturally-derived products. Several illustrative examples have been provided of processing concepts and equipment, from the laboratory scale through production plants. There now exists an extensive thirty year history of critical fluid processing technology upon which to draw, replete with many examples of components having nutraceutical value that have been already extracted, fractionated and reacted in these dense fluids.

In the future, fine particle production or food material modifications via critical fluid technology will join the other generic processing methods that involve the use of these novel fluids. As noted by King [66], an "all-green" processing platform is emerging involving the use of multiple environmentally-benign fluids and combined unit processing applications. Such an integrated critical fluid processing platform will make maximum use of production scale equipment, which undoubtedly will include facilities for particle production. Tailored towards the nutraceutical marketplace.

REFERENCES

[1] SS Koseoglu. Overview of nutraceuticals /functional foods. Presented at the Nutraceuticals and Functional Foods Short Course, College Station, Texas, February 24. 2000.

[2} JW King and GR List, eds. Supercritical Fluid Technology in Oil and Lipid Chemistry. Champaign, IL: AOCS Press, 1996.

[3] M Mukhopadhyay. Natural Extracts Using Supercritical Carbon Dioxide. Boca Raton, FL:CRC Press, 2000.

[4] JW King. Supercritical fluid processing of cosmetic raw materials. Cosmet. Toil. 61:61-67, 1991.

[5] E Revercon. Fractional separation of of SCF extracts from marjorum leaves: Mass transfer and optimization. J. Supercrit. Fluids 5:256-261, 1992.

[6] VJ Krukonis, JE Vivian, CJ Bambara, WB Nillson, and RA Martin. Concentration of eicosapentaenoic acid by supercritical fluid extraction: A design study of a continuous production process. In: GJ Flick and RE Martin, eds. Advances in Seafood Biochemistry. Basel:Technomic Publishing Company, 1992, pp. 169-179.

[7] E Reverchon, A Marciano, and M Poletto. Fractionation of peel oil key mixture by supercritical CO_2 in a continuous tower. Ind. Eng. Chem. Res. 36:4940-4948, 1997.

[8] JW King Sub- and supercritical fluid processing of agrimaterials: extraction, fractionation, and reaction modes. In: E. Kiran, ed. Supercritical Fluids – Fundamentals and Applications. Dordrecht:Kluwer, 2000, pp. 451-488.

[9] RS Mohamed, MDA Saldana, FH Socantatype, and TG Kieckbusch. Reduction in the cholesterol content of butter oil using supercritical ethane extraction and adsorption on alumina. J. Supercrit. Fluids 16:225-233, 2000.

[10] T Giese, M Johanssen, and G. Brunner Separation of stereoisomers in a SMB-SFC plant: Determination of isotherms and simulation. In: N. Dahmen and E. Dinjus, eds. Proceedings of the International Meeting of the GVC-Fachausschuss Hochdruckverfahrenstechnik, Karlsruhe, Germany, pp. 283-286.

[11] RJ Nicolosi, LM Ausman, and EJ Rogers. In: New Technologies for Healthy Foods & Nutraceuticals, Shrewsbury, MA:ATKL Press, Inc., 1007, p. 84.

[12] KC Fitzpatrick. Sterol ester products face US hurdles. INFORM. 10:172-176, (1999).

[13] PT Anastas, and Williamson, T.C., eds. Green Chemistry, Washington, DC, American

Chemical Society, 1996.

[14] GW Froning, RL Wehling, SL Cuppett, MM Pierce, .L Niemann, and DK Siekman. Extraction of cholesterol and other lipids from dried egg yolk using supercritical carbon dioxide. J. Food Sci. 55:95-98, 1990.

[15] CA Passey. Commercial feasibility of a supercritical extraction plant for making reduced-calorie peanuts. In: S.S.H. Rizvi , ed. Supercritical Fluid Processing of Food and Biomaterials. London: Blackie Academic, 1994, pp. 223-243.

[16] E Stahl, K-W Quirin, and D Gerard. Dense Gases for Extraction and Refining, Heidelberg, Germany:Springer-Verlag, 1987.

[17] Anonymous. CO_2 extracts - food and medicine. Food Marketing Technol. 11(4):27-31, 1997.

[18] JW King, JL Johnson, WL Orton, FK McKeith, PL O=Connor, J Novakofski, and TR Carr. Effect of supercritical carbon dioxide extraction on the fat and cholesterol content of beef patties. J. Food Sci. 58: 950-952,958, 1993.

[19] AA Clifford, A Basile, MM Jimenez-Carmona, M.M., and SHR Al-Said. Extraction of natural products with superheated water. *In:* Proceedings of the GVC-Fachaussschuss Hochdruckverfahrenstechnik. Karlsruhe, Germany, 1999, p. 181-184.

[20] M Mannia, H Kim, and CM Wai. Supercritical carbon dioxide and high pressure water extraction of bioactive compounds in St. John's wort. Proceedings of the Super Green 2002 Symposium, Suwon, South Korea, 2002, pp 74-78.

[21] JW King, RD Grabiel, and JD Wightman. Subcritical water extraction of anthocyanins from fruit berry substrates. Proceedings of the 6th International Symposium on Supercritical Fluids – Vol. 1. Versailles, 2003, pp. 409-418.

[22] MB King and TR Bott, eds. Extraction of Natural Products Using Near-Critical

Solvents, London:Blackie Academic, 1993.

[23] H Brogle, J. CO_2 as a solvent: its properties and uses. Chem. Ind. 19:385-390, 1982.

[24] AA Clifford, A Basile, A., MM Jiminez-Carmona, M.M., and SHR Al-Saidi. Extraction of natural products with superheated water. In: Proceedings of the 6th Meeting on Supercritical Fluid Chemistry and Materials, Nancy, France., 1999, pp. 485-490.

[25] A Basile, MM Jimenez-Carmona, and AA Clifford. Extraction of rosemary by superheated water. J. Agric. Food. Chem. 46: 5205-5209, 1998.

[26] SB Hawthorne, Y Yang, and DJ Miller. Extraction of organic pollutants from environmental solids with sub- and supercritical water. Anal. Chem. 66: 2912-2920, 1994.

[27] JW King. Critical fluid options for isolating and processing agricultural and natural products. In: Proceedings of the Super Green 2002 Symposium, Suwon, South Korea, 2002, pp 74-78.

[28] JW King, FJ Eller, SL Taylor, and AL Neese. Utilization of analytical critical fluid instrumentation in non-analytical applications. Proceedings of the 5th International Symposium on Supercritical Fluids. Atlanta, GA, (CD Disk).

[29] A Pietsch, and R Eggers. The mixer-settler principle as a separation unit in supercritical fluid processes. J. Supercrit. Fluids 14: 163-171, 1999.

[30] WB Nilsson. Supercritical fluid extraction and fractionation of fish oils. In: JW King and GR List, eds. Supercritical Fluid Technology in Oil and Lipid. Champaign, IL: AOCS Press, USA, 1996, pp. 180-212..

[31] JW King, F Favati, and SL Taylor. Production of tocopherol concentrates by supercritical fluid extraction and chromatography. Sep. Sci. Tech. 31:1843-1857, 1996.

[32] S Peper, S Cammerer, M Johannsen, and G Brunner. Supercritical fluid chromatography process optimization of the separation of tocopherol homolgues. In: Proceedings of the 6th International Symposium on Supercritical Fluids. Versailles, France, 1999, pp. 563-568.

[33] R Eggers. High pressure extraction of oilseeds. J. Am. Oil. Chem. Soc. 62:1222-1230, 1985.

[34] JW King. Analytical-process supercritical fluid extraction: a synergistic combination for solving analytical and laboratory scale problems. Trends Anal. Chem. 14:474-481, 1995.

[35] JW King, J.W. Critical fluids for oil extraction. In: PJ Wan and PJ Wakelyn (Eds.) Technology and Solvents for Extracting Oilseeds and Non-petroleum Oils. Champaign, IL:AOCS Press, 1997, pp. 283-310.

[36] GR List, JW King, JH Johnson, K Warner, and TL Mounts. Supercritical CO_2 degumming and physical refining of soybean oil. J. Am. Oil Chem. Soc. 70:473-477, 1993,.

[37] SL Taylor, JW King, L. Montanari, P Fantozzi, and MA Blanco. Enrichment and fractionation of phospholipid concentrates by supercritical fluid extraction and chromatography. Italian J, Food Sci. 12:65-76, 1999.

[38] WG Schultz and JM Randall. Liquid carbon dioxide for selective aroma extraction. Food Tech. 24(11): 94-98, 1970.

[39] JR Robinson and M Sims. Method and system for extracting a solute from a fluid using dense gas and a porous membrane. U.S. Patent 5,490,884 (1996).

[40] ML Lee and KE Markides, eds. Analytical Supercritical Fluid Chromatography and Extraction, Provo, UT:Chromatography Conferences, 1990.

[41] LT Taylor. Supercritical Fluid Extraction, John Wiley & Sons, New York, NY,

1998.

[42] MD Luque de Castro, M Valcarel, .and MT Tena. Analytical Supercritical Fluid Heidelberg: Springer-Verlag, 1994.

[43] JR Dean, ed. Applications of Supercritical Fluids in Industrial Analysis, London:Blackie Academic, 1993.

[44] JW King,. Applications of capillary supercritical fluid chromatography-supercritical fluid extraction to natural products. J. Chromatogr. Sci. 28: 9-14, 1990.

[45] JW King. Capillary supercritical fluid chromatography of cosmetic ingredients and formulations. J. Microcol. Sep. 10:33-39, 1998 .

[46] A Chandra and MG Nair. Supercritical carbon dioxide extraction of daidzein and genistein from soybean products. Phytochem. Anal. 7:259-262, 1996.

[47] L Montanari, P Fantozzi, JM Snyder, and JW King, J.W. Selective extraction of phospholipids from soybeans with supercritical carbon dioxide and ethanol. J. Supercrit. Fluids 14: 87-93, 1999..

[48] SL Taylor, and JW King. Supercritical luid fractionation of phospholipids. In: Abstracts of the 8th Int. Symposium on Supercritical Fluid Chromatography and Extraction., St. Louis, Missouri, 1998.

[49] SL Taylor, JW King, and JM Snyder. Tandem supercritical fluid extraction/ chromatographic studies of the desert botanical species, Dalea spinosa. J. Microcol. Sep. 6: 467-473, 1994.

[50] JW King and JM Snyder, J.M. Supercritical fluid chromatography: a shortcut in lipid analysis. In: RE McDonald and M Mossoba, eds. New Techniques and Applications in Lipid Analysis. Champaign, Il:AOCS Press1997, pp. 139-162.

[51] JM Snyder, SL Taylor, and JW King, J.W.. Analysis of tocopherols by capillary supercritical fluid chromatography and mass spectrometry. J. Am. Oil Chem. Soc.

70: 49- 354, 1993.

[52] SI DeSwaef, W Kleibohmer, and AJ Vlietinck, A.J. Supercritical fluid chromatography of free fatty acids and ethyl esters in ethanolic extracts of sabal serrulata. Phytochem. Anal. 7: 223-227, 1996.

[53] F Favati, JW King, JP Friedrich, and K Eskins. Supercritical extraction of carotene and lutein from leaf protein concentrates. J. Food Sci. 53: 1532-1536, 1988.

[54] D Ssuss, and G Brunner, Countercurrent extraction with supercritical carbon dioxide: behaviour of a complex natural mixture. In: Proceedings of the GVC-Fachaussschuss Hochdruckverfahrenstechnik., Karlsruhe, Germany, 1999, pp. 189-192.

[55] JW King, GR List, and JH Johnson, J.H.. Supercritical carbon dioxide extraction of spent bleaching clays. J. Supercrit. Fluids 5: 38-41, 1992.

[56] Authors in Table 8, In: Proceedings of the 6th International Symposium on Supercritical Fluids – Tome 3, Versailles, France, 2003, Pages in Table 8.

[57] R Eggers and H Wagner. Extraction device for high viscous media in a high-turbulent two-phase flow with SC-CO_2. J. Supercrit. Fluids 6:31-37, 1993.

[58] H. Wagner and R. Eggers. Entolung von sojalecithin mit uberkritischen kohlendioxid in hochturbulenter zweiphasestromung. Fat Sci. Technol. 95:75-80, 1993.

[59] TP Castor and L Chen. Method and apparatus for making liposomes containing hydrophobic drugs .US Patent 5, 776, 486, July 7, 1998.

[60] TP Castor. Methods and apparatus for making liposomes. US Patent 5,554, 382, September 10, 1996.

[61] C Magnan, H Commenges, E Badens ,and G Charbit. Fine phospholipid particles formed by precipitation with a compressed fluid anti-solvent. Proceedings of the GVC-Fachaussschuss Hochdruckverfahrenstechnik., Karlsruhe, Germany,1999, pp.

223.

[62] C Magnan, E Badens, N Commenges, and G Charbit. Soy lecithin micronization by precipitation with a compressed fluid anti-solvent – influence of process parameters. J. Supercrit. Fluids 19:69-77, 2000.

[63] A Weber, C Nolte, M Bork, and R Kummel. Recovery of lecithin from egg yolk-extracts by gas anti-solvent crystallization. In: Proceedings of the 6th Meeting on Supercritical Fluids: Chemistry and Materials. Nottingham, England, pp. 181-184.

[64] L Frederiksen, K Anton, BJ Barrat, P VanHoogevest, and H Lenenberger. In: Proceedings of the 3rd International Symposium on Supercritical Fluids – Tome 3, Strasbourg, France, 1994. pp. 235-240.

[65] J Heidlas and Z. Zhang. New approaches to formulate compounds using supercritical gases. In: Proceedings of the 7th Meeting on Supercritical Fluids – Tome 1. Antibes, France, 2000, pp. 167-172.

{66] JW King. Critical fluid options for isolating and processing agricultural and natural products. Proceedings of SuperGreen 2002. Suwon, South Korea, 2002, pp. 61-66.

Table 1. Nutraceutical and their therapeutic use and current production via critical fluids.

Nutraceutical	Utility	Processed Via Critical Fluids
Saw Palmetto	Prostate	Yes
Kava-Kava	Anxiolytic	No
Hawthorne	Cardiotonic	No
Ginseng	Tonic	Yes
Garlic	Circulatory	Yes
Ginko Biloba	Cognitive	No
St. John=s Wort	Depression	No
Chamomile	Dermatological	Yes
Echinacea	Colds/Flu	Yes
Black Cohosh	Gynecological	No
Lutein	Macular Degeneration	Yes
Flavanoids	Anti-Cancer	No
Isoflavones	PMS, Circulatory	No
Omega 3 EFA, DHA	Circulatory	Yes
Evening Primrose	Inflammation	Yes
Phytosterols	Circulatory	Yes
Tocopherols	Antioxidant	Yes
Phospholipids	Cognitive	Yes

Table 2. Natural oils extracted with critical fluids and their nutraceutical components.[1]

Natural Oils	Nutraceutical Component
Rice bran	n-6, n-3 Fatty acids
Safflower	Phystosterols
Marine	Tocopherols
Sesame	Carotenoids
GLA-enriched	Phospholipids
Oat	Tocotrienols
Almond	Oryzanol
Wheat germ	Sesamolin
Amaranth	Glycolipids
Essential	Conjugated fatty acids
Avocado	Lipoproteins
Grape Seed	
Macadamia nut	
Kiwi	
Genetically-modified oils	

[1] The nutraceutical components listed on the same line as a particular oil does not mean that nutraceutical moiety is associated with that oil.

Table 3. - Enrichment factors of tocopherols from soybeans by SFE and SFE/SFC.

Tocopherol	SFE	SFE/SFC
alpha	4.33	12.1
beta	1.83	2.4
gamma	3.94	15.0
delta	3.75	30.8

Table 4. - Relative amount of phospholipids from soybeans in SFE isolates and in SFC collected fractions.

Phospholipid	SFE [a]	SFC
Phosphatidylethanolamine	16.1	76.8
Phosphatidylinositol	9.2	74.9
Phosphatidic Acid	2.8	20.8
Phosphatidylcholine	15.6	55.8

All data in percent of that component

[a] relative to other eluting constitutents (oil and unidentified peaks)

Table 5 - Enrichment of aroma constituents by the Porocrit process.

Compound	ppm in feed	Conc. Factor in Extract	% Depletion
Methanol	4,220	3	-
Ethanol	105,000	7	20
1 - Propanol	270	82	69
Amyl Alcohol	17	115	81
Hexanol	3	109	95+
Octanol	5	110	95+
Z-3-Hexanol	10	127	95+
α - terpinenol	10	98	95+
Terpinen-4-ol	4	106	95+
Ethyl Acetate	29	106	85
Ethyl Butyrate	23	106	88
Acetal	37	147	39
ε -2- Hexenal	19	132	86
Hexanal	10	100	84
Octanal	5	156	95+
Citronellal	3	62	95+

Table 6. - Critical fluid analytical technology - relevance to nutraceutical product development.

To indicate solubility or extractability of a compound
For fractionating a natural product
In support of process development
For analysis of critical fluid-derived extract
To deformulate a commercial product
To determine required physicochemical data

Table 7. - SFC fractionation of lecithin on silica gel.

Fraction Collected	Eluent Parameters	Predominate Compounds
#1	350 bar, 50°C, CO_2	Triglyceride Oil
#2	350 bar, 50°C, CO_2/M	A
#3	350 bar, 50°C, CO_2/M	
#4	500 bar, 50°C, CO_2/M	Phosphatidylethanolamine
#5	500 bar, 80°C, CO_2/M	Phosphatidylinositol + Phosphatidycholine
#6	500 bar, 80°C, CO_2/M	Phosphatidylcholine
#7	500 bar, 80°C, CO_2/M	Phosphatidylcholine

Fraction #2 modifier is 10% ethanol:water (9:1)

Fractions #3-7 modifier is 25% ethanol:water (9:1)

Table 8. Organizations processing or offering critical fluid-derived products.

Flavex (Germany)	Fuji Flavor (Japan)
Hermsen (Germany)	Kobe (Japan)
HVG Barth (Germany)	Mori Oil Mills (Japan)
Kaffe HAG (Germany)	Ogawa (Japan)
SKW Trostberg (Germany)	Takasago (Japan)
KD - Pharma - IQA (Germany-Spain)	Takeda (Japan)
General Foods (United States)	Cultor (France)
John Haas (United States)	HITEX/Separex (France)
Praxair (United States)	Norac (Canada)
Yakima Chief (United States)	Aroma Tech OY (Finland)
Carlton United Breweries (United Kingdom)	Quest (Holland)
English Hops (United Kingdom)	Wells Investment Ltd. (New Zealand)
Steiner Hops (Germany-United Kingdom-USA)	Lavipharm (Greece/USA)
US Nutraceuticals (United States)	GreenTek 21 (South Korea)
Supertrae (Denmark)	Eiffel (Australia)
Ferro Corporation (United States)	Arkopharma (France)

Table 9. Materials processing using critical fluid media with implication or direct application in the nutraceutical or functional food industry.

Process	Application	Reference[1]
Concentrated Powder Form (CPF), Jet Dispersion	Dispersion of Paparika Soup Powders	E. Weidner pp. 1483-1495
Semi-continuous Gas Antisolvent Process	Cholesterol Morphology and Precipitation	P. Subra/A. Vega pp. 1629-1634
Rapid Expansion of Supercritical Solution	Phystosterol Micronization	S. Jiang et al pp. 1653-1658
DELOS Crystallization	Crystallization and Control of Stearic Acid Morphology	N. Ventosa et al pp. 1673-1676
CPF Process	Controlled Release of Flavors And Vitamins	F. Otto et al. pp. 1707-1712
Rapid Expansion of Supercritical Solution	Encapsulation of β-sitosterol in Low MW Polymer Matrix	M. Turk et al. pp. 1747-1752
Supercritical Anti-Solvent Process	Incorporation of cholesterol or proteins in biodegradable matrix	Pellikaan pp. 1765-1770
PGA & GAS Processes	β-Carotene Precipitation	F. Miguel et al. pp. 1783-1788
Particle from Gas Saturated Solution (PGSS)	Lipid Micronization of Phosphatidylcholine and Tristearin	N. Elvassore et al. pp. 1853 – 1858
Supercritical Anti-Solvent Process	Biodegradable Polymers Precipitation Studies with	A Vega-Gonzalez pp. 1877-1882

[1] The listed authors and inclusive page numbers all appear in the same reference (#56) in the reference section.

Figure Captions:

Fig. 1 - Phase diagram of water.

Fig. 2 - AGreen@ critical fluid processing options.

Fig. 3 - Separation scheme for nutraceutical components in soybeans.

Fig. 4 - Bench scale supercritical fluid extraction system.

Fig. 5 - Continuous recycle supercritical fluid extractor.

Fig. 6 - Semi-continuous pilot plant extraction system.

Fig. 7 - Continuous countercurrent refining system.

Fig. 8 - Thermal gradient supercritical fluid fractionation column.

Fig. 9 - Tocopherol enrichment/fractionation by SFE/SFC technique.

Fig. 10 - Porocrit continuous fluid extraction process.

Fig. 11 - Capillary SFC characterization of deodorizer distillate feedstock..

Fig. 12 - Capillary SFC analysis of capsule containing sawtooth palmetto berry extract..

Fig. 13 – Basic schematic of laboratory scale jet extractor system. PRV = pressure regulating valve; T = flow totalizer.

Fig. 13a – Details of jet extractor for deoiling soya lecithin. BPRV = back pressure regulating valve; T flow totalizer.

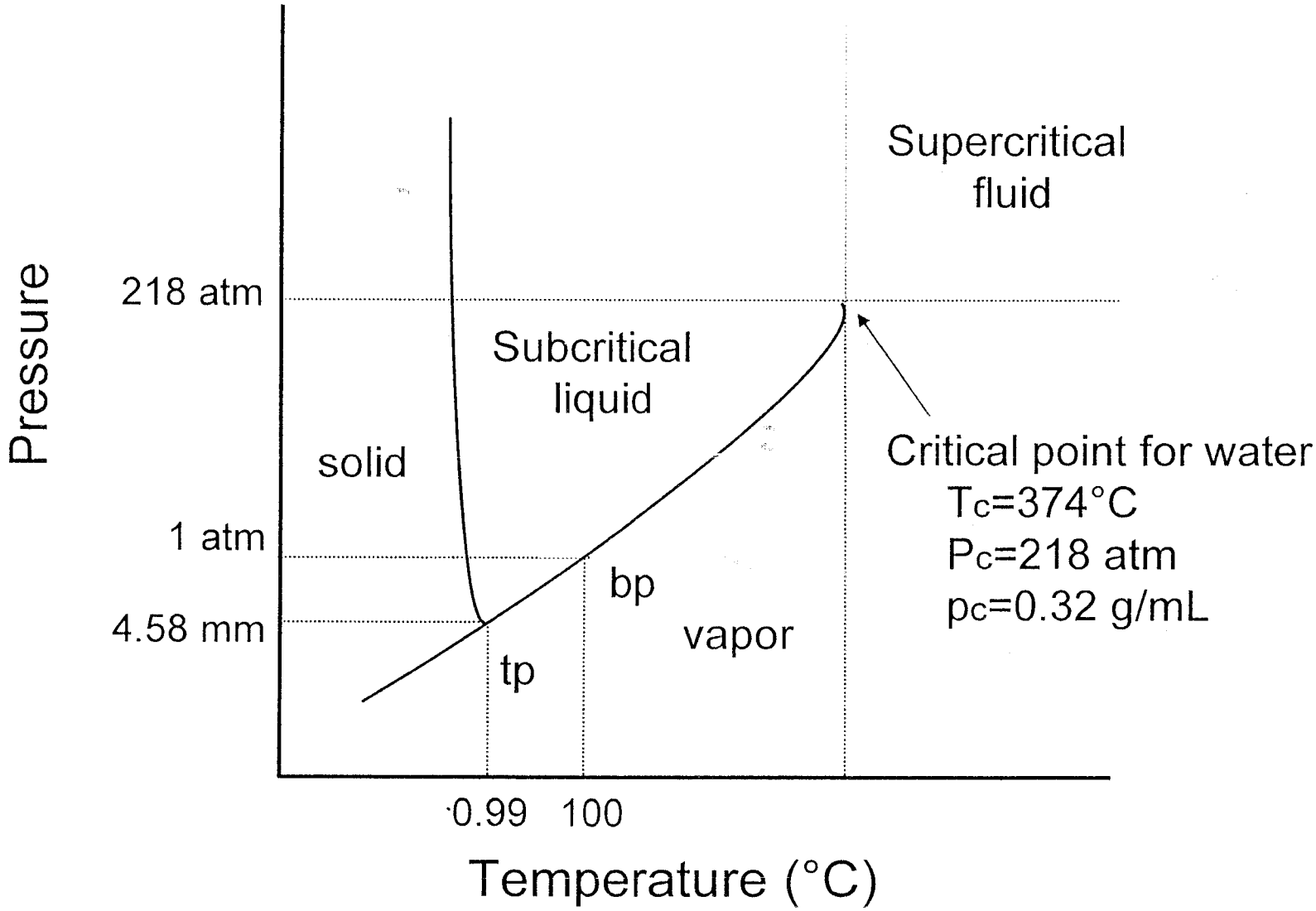

Pressure
218 atm
1 atm
4.58 mm
Supercritical
fluid
Subcritical
liquid
solid
Critical point for water
Tc=374°C
Pc=218 atm
pc=0.32 g/mL
bp
vapor
tp
0.99
100
Temperature (°C)

Fig 1

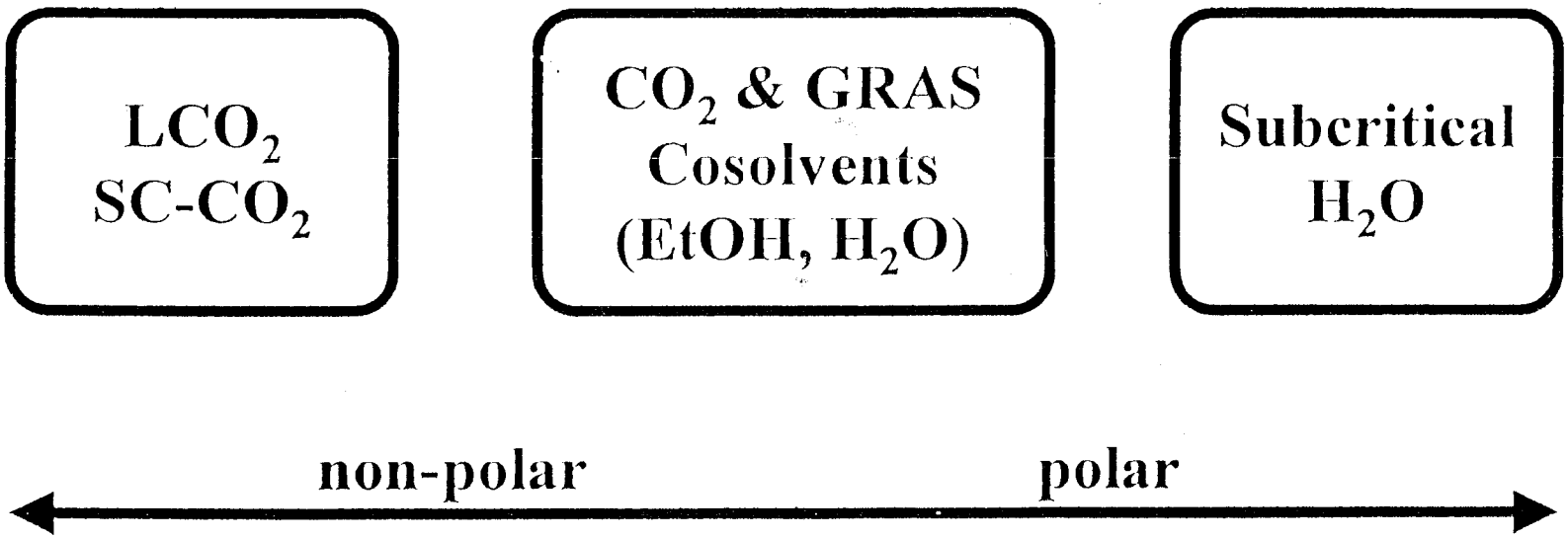
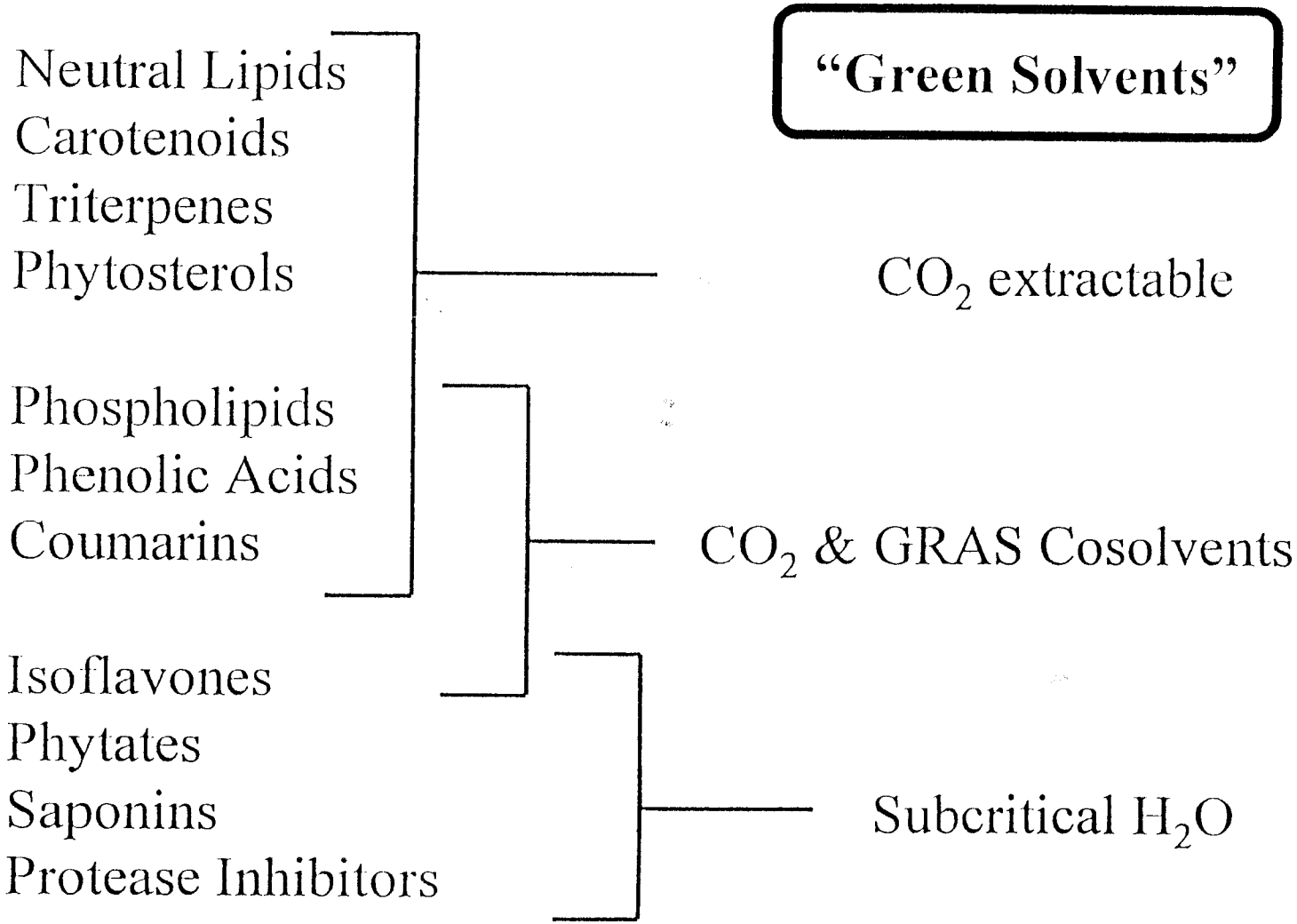
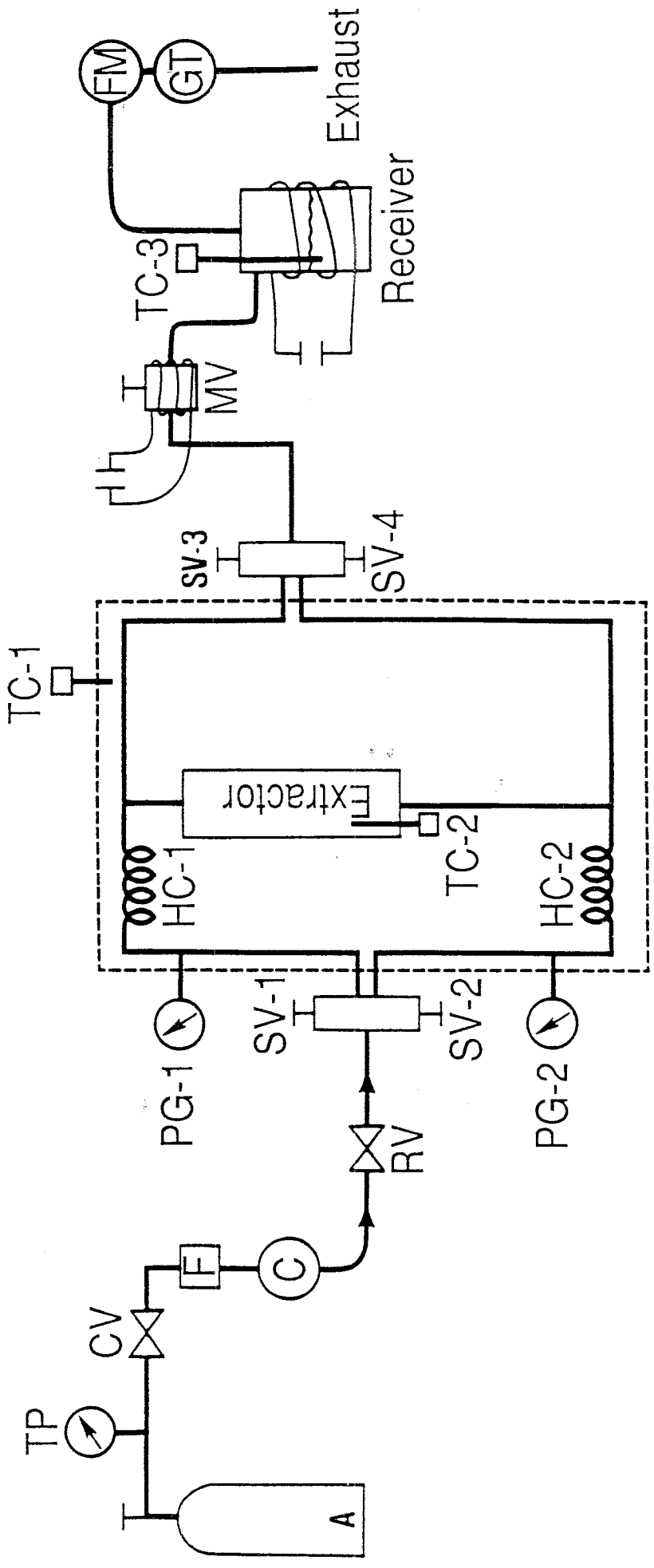
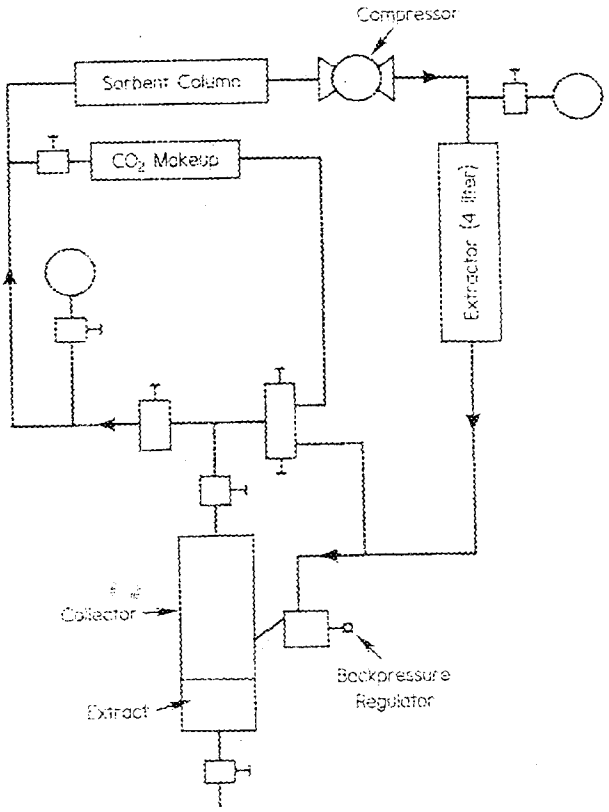
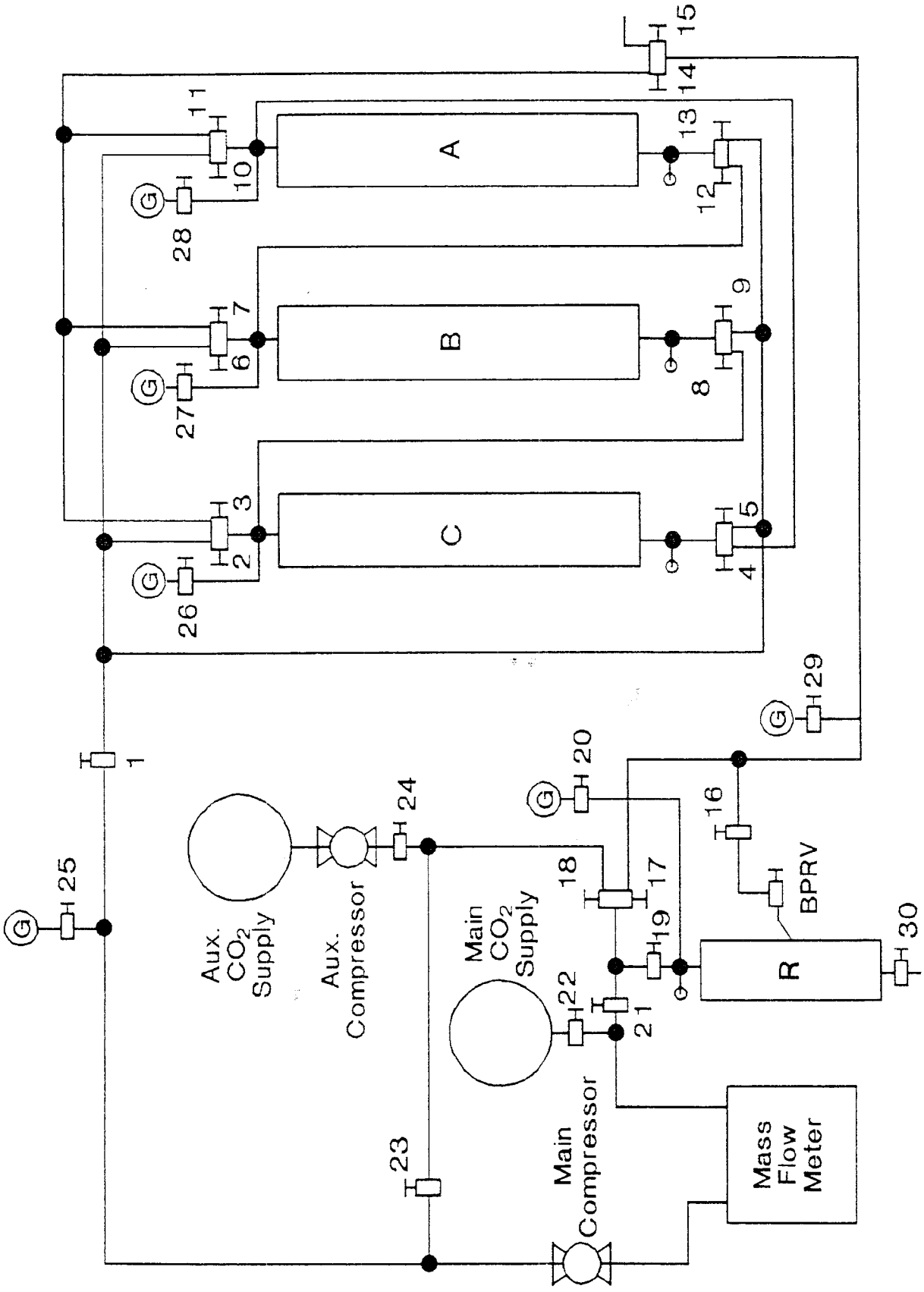

LCO_2
$SC\text{-}CO_2$
CO_2 & GRAS
Cosolvents
(EtOH, H_2O)
Subcritical
H_2O
non-polar
polar

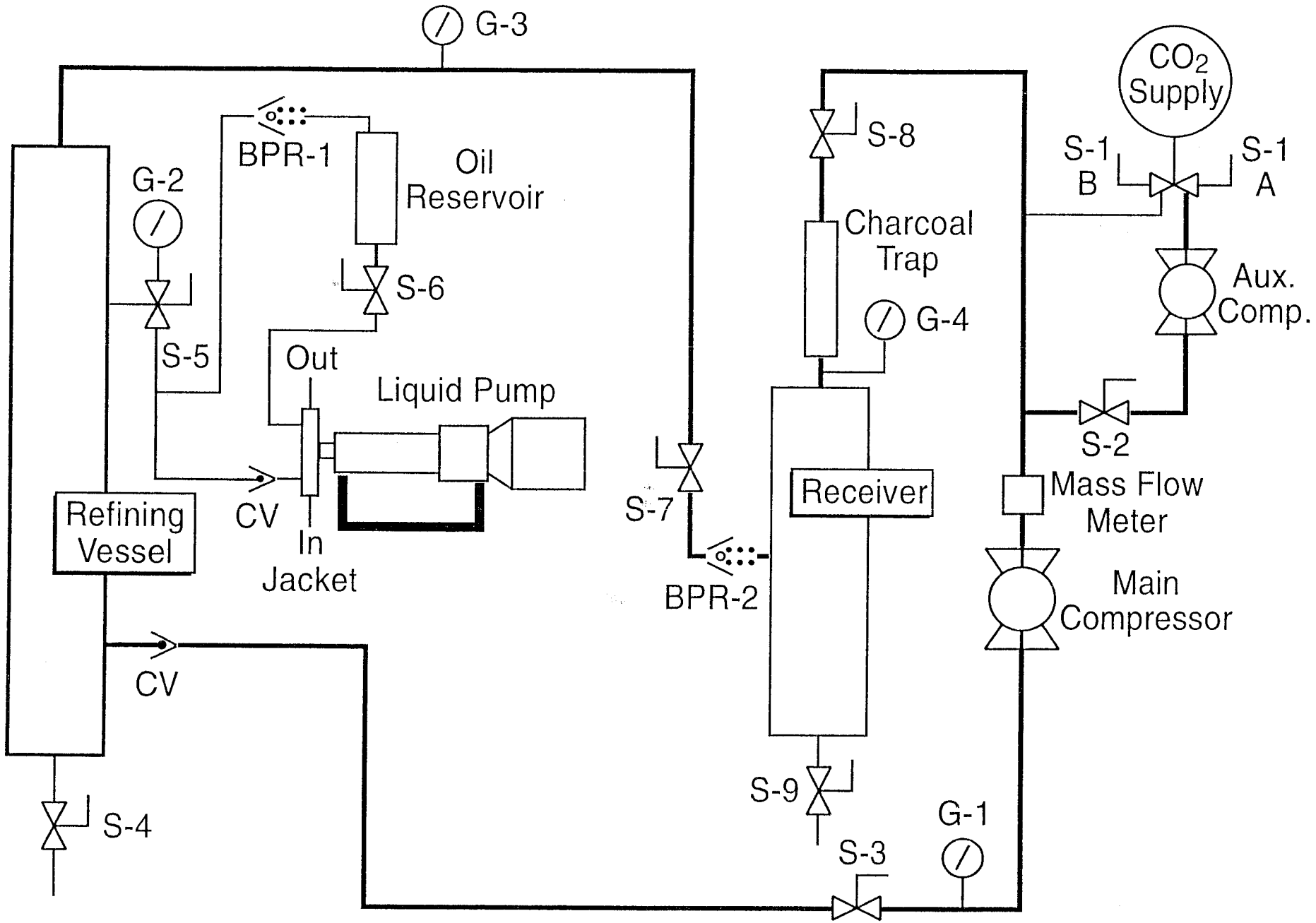
Fig. 2

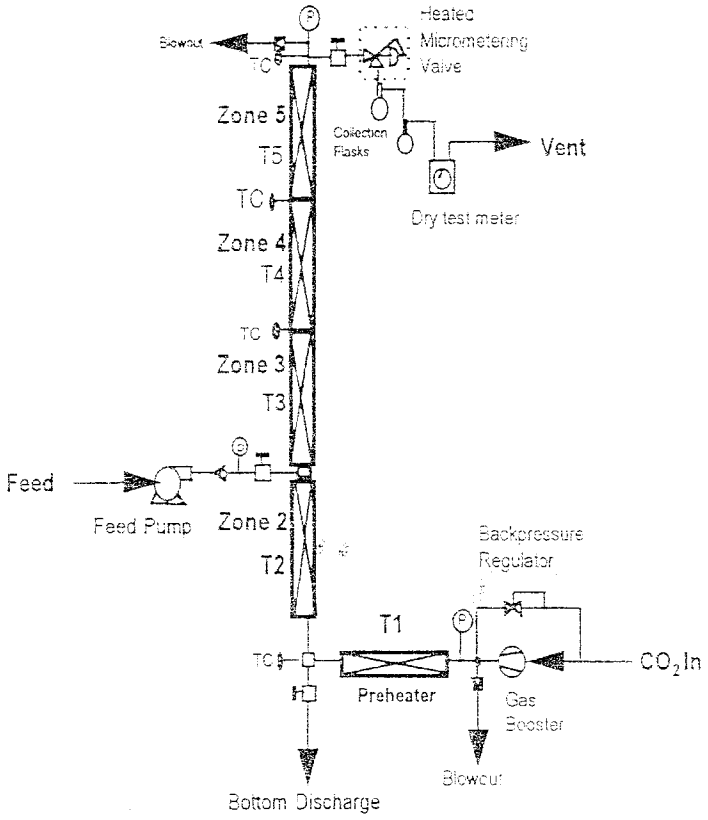

"Green Solvents"
Neutral Lipids
Carotenoids
Triterpenes
Phytosterols
CO_2 extractable
Phospholipids
Phenolic Acids
Coumarins
CO_2 & GRAS Cosolvents
Isoflavones
Phytates
Saponins
Protease Inhibitors
Subcritical H_2O

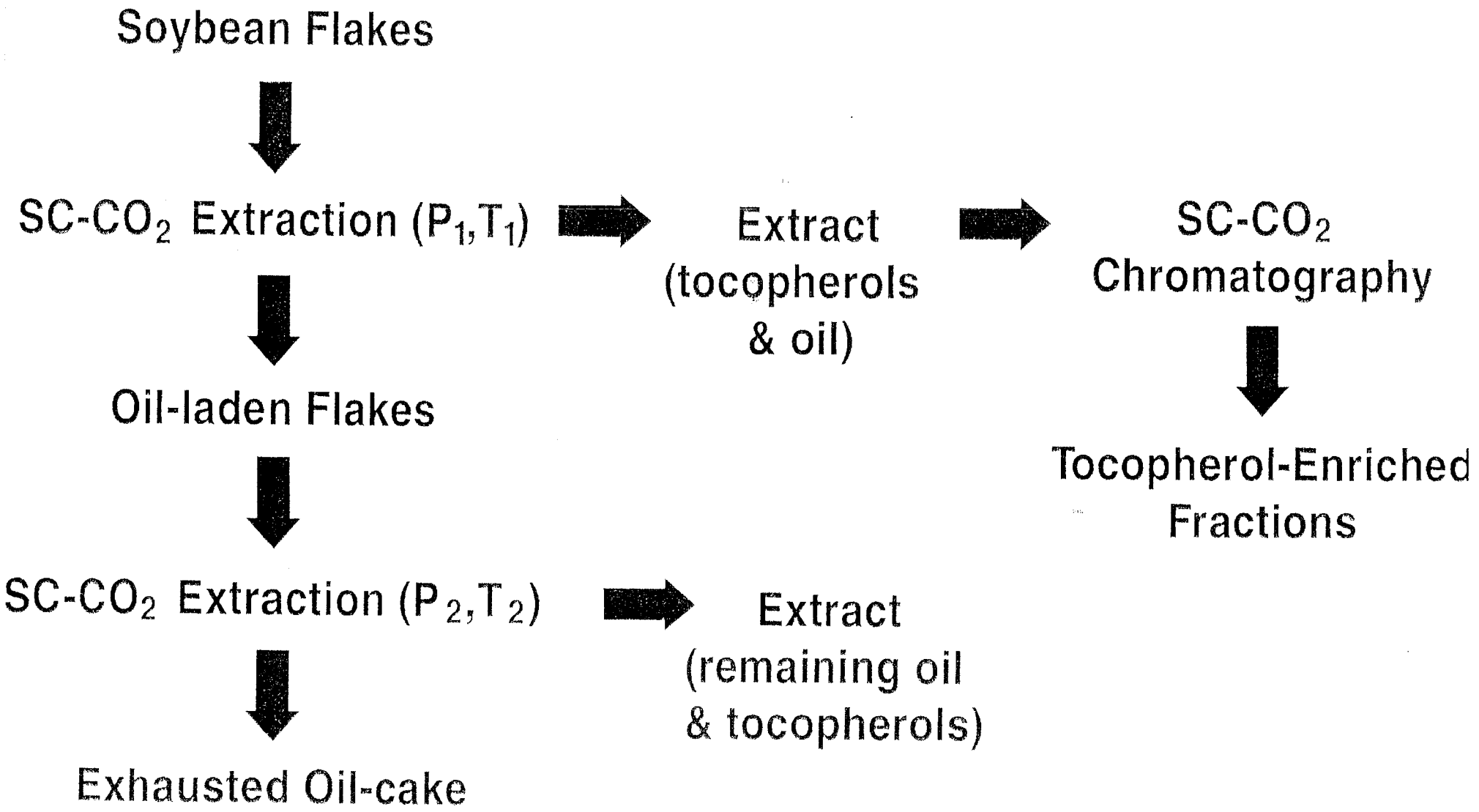

FM
GT
Exhaust
Receiver
TC-3
MV
SV-3
SV-4
TC-1
Extractor
HC-1
TC-2
HC-2
SV-1
SV-2
PG-1
PG-2
RV
F
C
CV
TP
A


Compressor
Sorbent Column
CO_2 Makeup
Extractor (4 liter)
Collector
Extract
Backpressure
Regulator

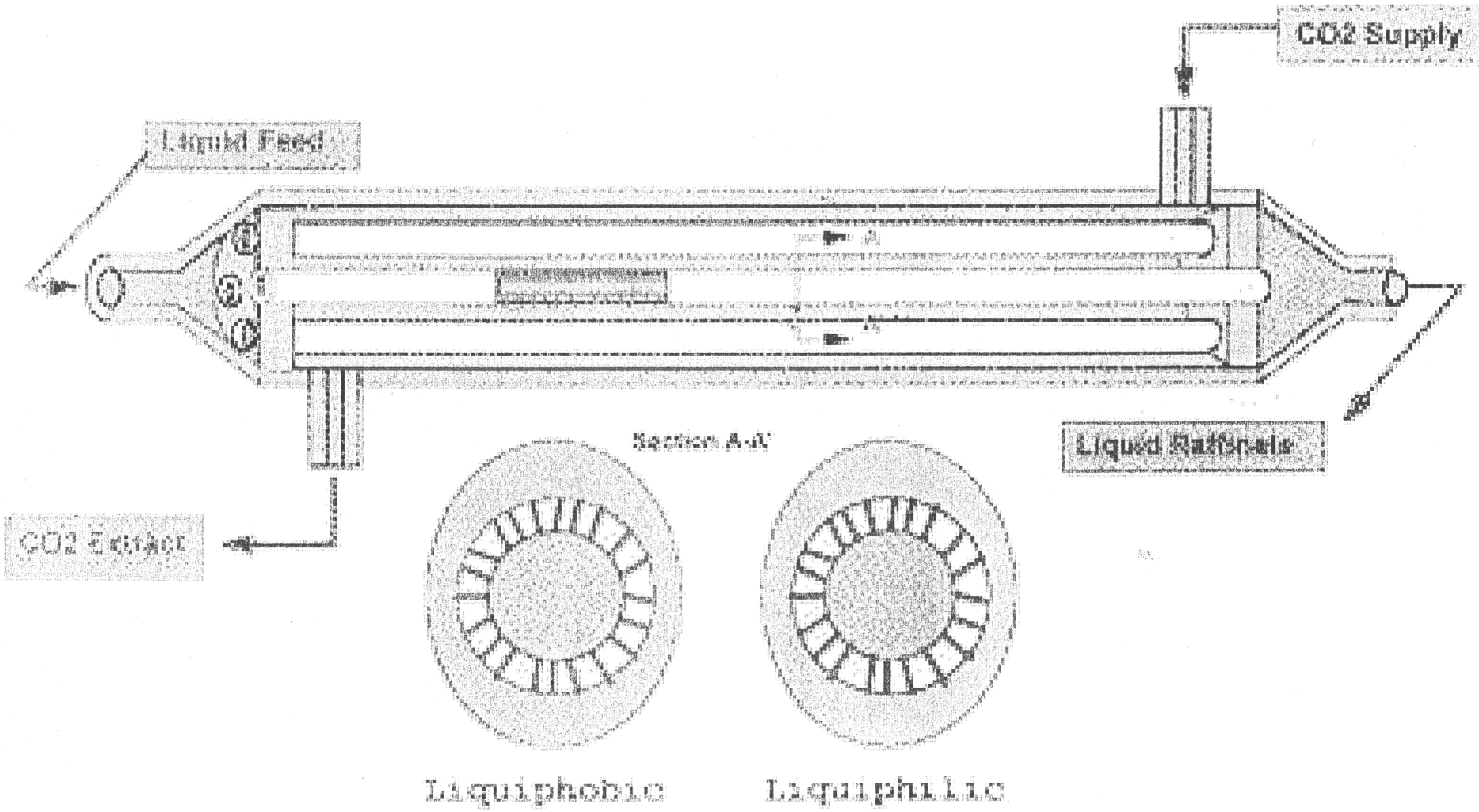

A
B
C
R
BPRV
Aux. CO_2 Supply
Aux. Compressor
Main CO_2 Supply
Main Compressor
Mass Flow Meter
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

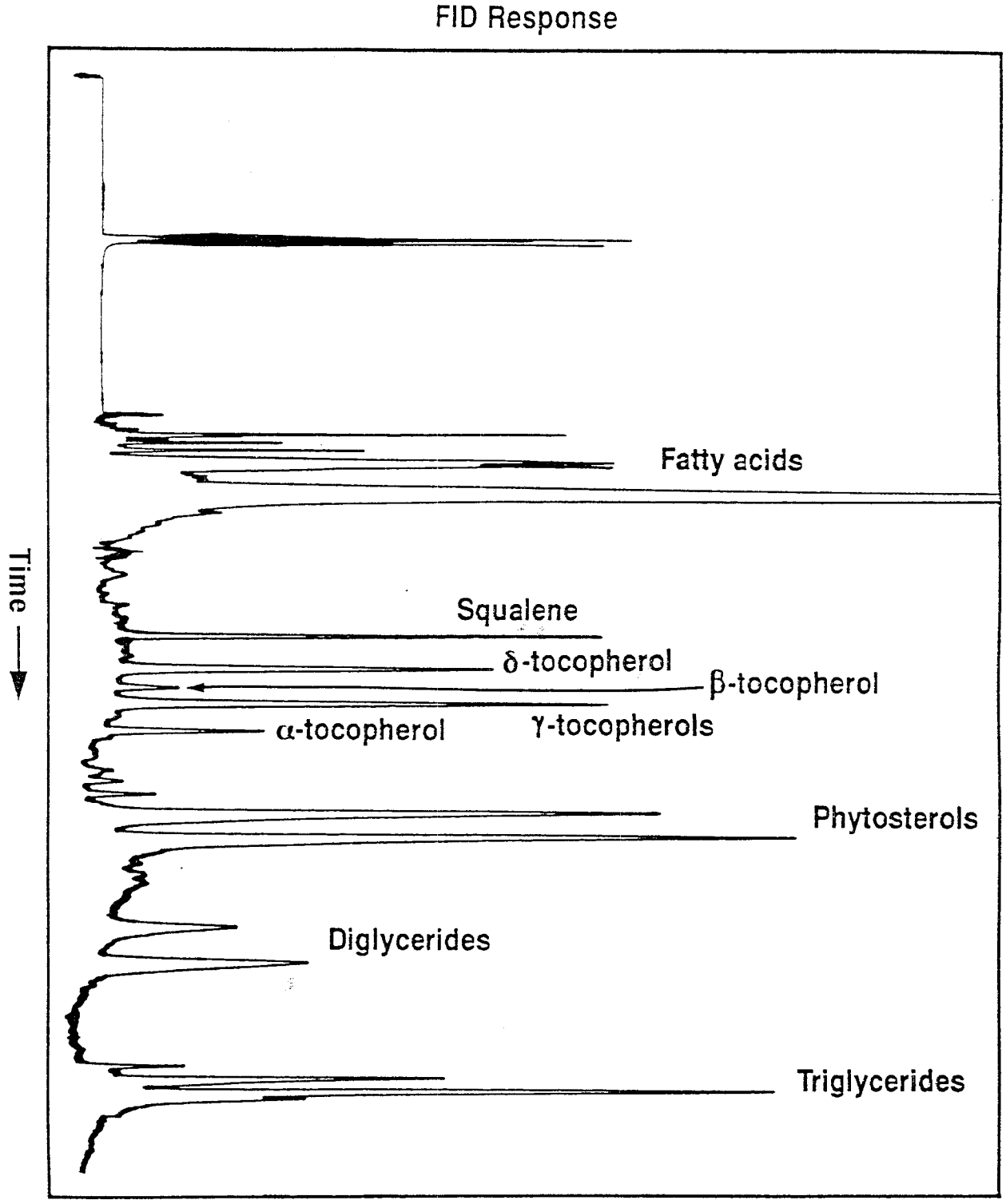

G-3
CO_2 Supply
S-8
S-1 B
S-1 A
BPR-1
Oil Reservoir
Charcoal Trap
G-2
S-6
G-4
Aux. Comp.
S-5
Out
Liquid Pump
S-2
Receiver
Mass Flow Meter
Refining Vessel
CV
In Jacket
S-7
BPR-2
Main Compressor
CV
S-9
G-1
S-4
S-3


Heated
Micrometering
Valve
Blowout
TC
Zone 5
T5
Collection
Flasks
Vent
Dry test meter
TC
Zone 4
T4
TC
Zone 3
T3
Feed
Feed Pump
Zone 2
T2
Backpressure
Regulator
T1
TC
Preheater
Gas
Booster
CO_2 In
Blowout
Bottom Discharge


Soybean Flakes
SC-CO2 Extraction (P1,T1)
Extract (tocopherols & oil)
SC-CO2 Chromatography
Tocopherol-Enriched Fractions
Oil-laden Flakes
SC-CO2 Extraction (P2,T2)
Extract (remaining oil & tocopherols)
Exhausted Oil-cake

Porocritical Membrane Contactor




FID Response
Time
Fatty acids
Squalene
δ-tocopherol
β-tocopherol
α-tocopherol
γ-tocopherols
Phytosterols
Diglycerides
Triglycerides

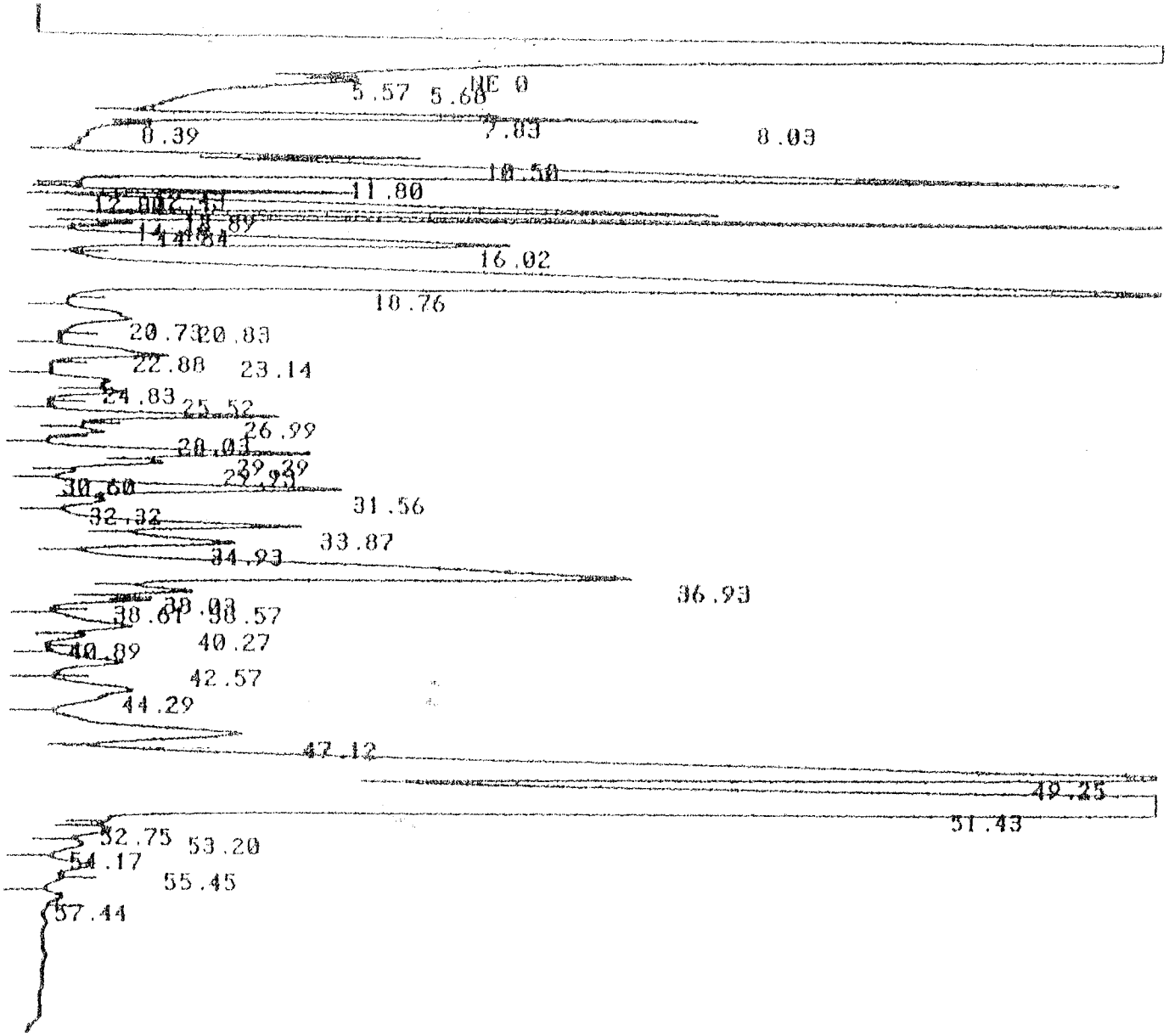
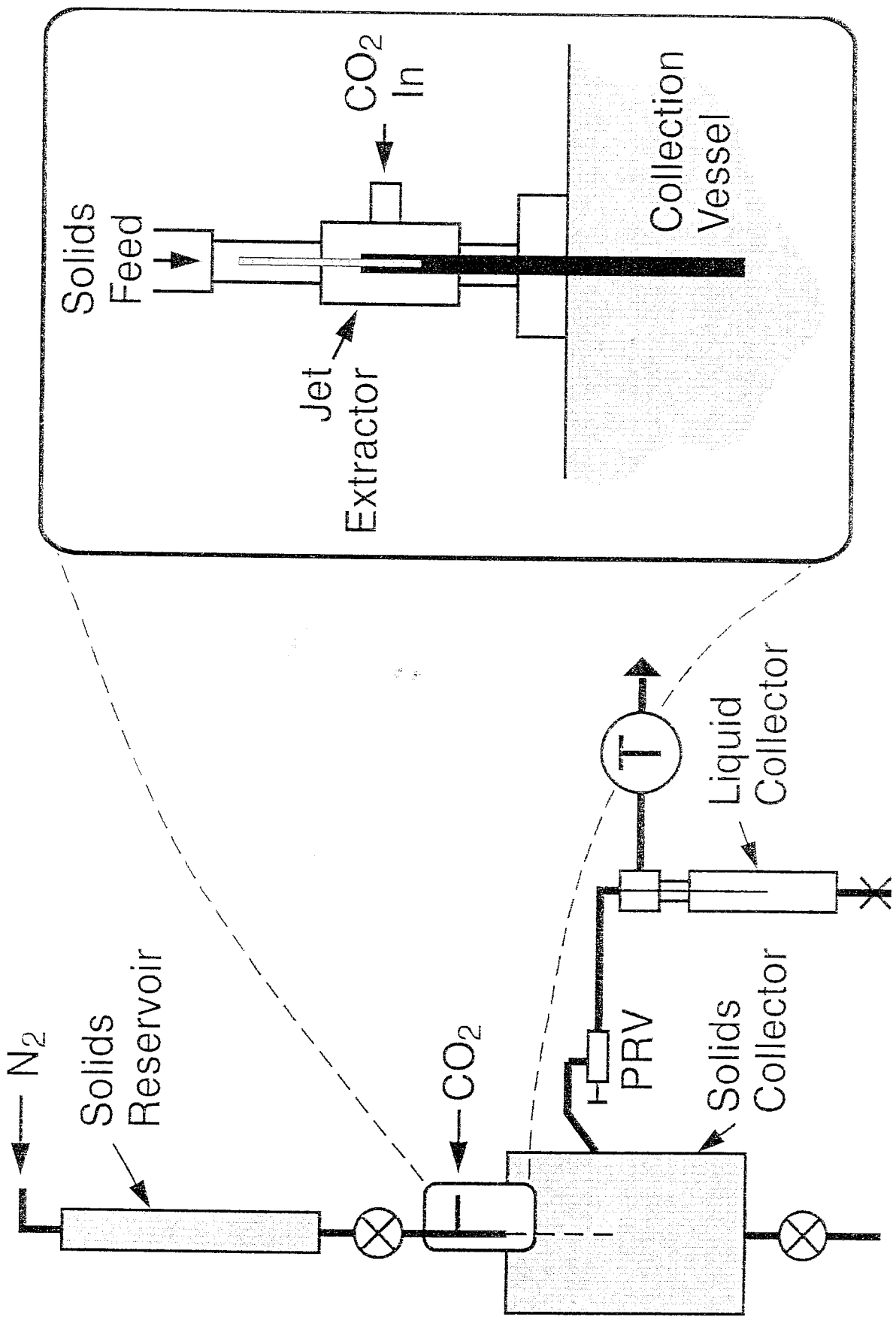
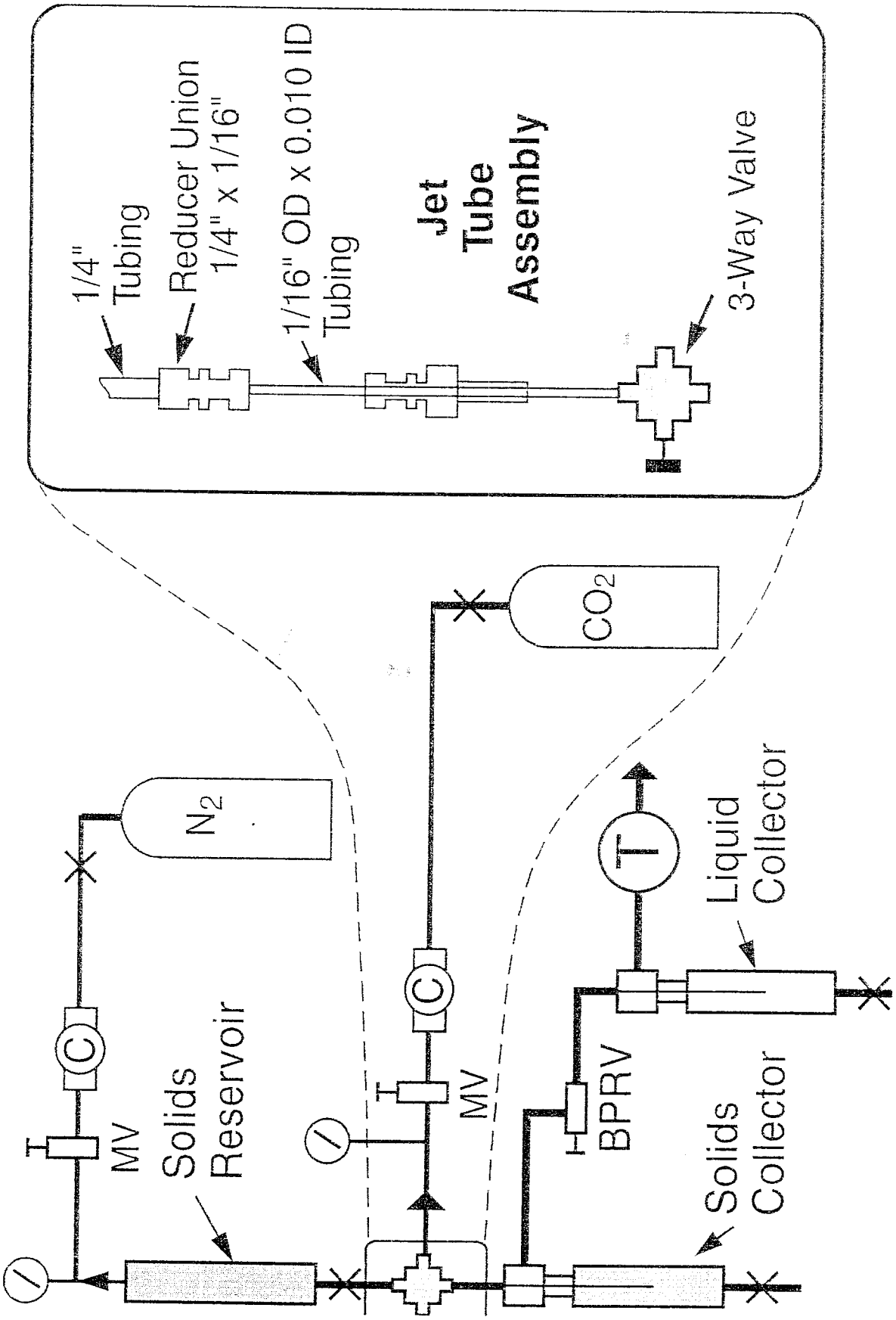

DE 0
5.57
5.68
0.39
7.83
8.03
10.50
11.80
16.02
10.76
20.73
20.83
22.88
23.14
24.83
25.52
26.99
30.60
31.56
32.32
33.87
34.93
36.93
38.61
38.57
40.89
40.27
42.57
44.29
42.12
49.25
51.43
52.75
53.20
54.17
55.45
57.44

FIG. 13


Solids Feed
CO_2 In
Collection Vessel
Jet Extractor
N_2
Solids Reservoir
CO_2
PRV
T
Liquid Collector
Solids Collector


1/4" Tubing
Reducer Union 1/4" x 1/16"
1/16" OD x 0.010 ID Tubing
Jet Tube Assembly
3-Way Valve
N_2
CO_2
MV
Solids Reservoir
MV
BPRV
Liquid Collector
Solids Collector